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Methodology Article

Isolation of Selected Bacterial Pathogens from Bovine Mastitis in Selected Dairy Farms Found in Dire Dawa Town, Eastern Ethiopia

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Abstract: Mastitis is a complex and costly dairy cattle disease. The main aim of this study to isolate bacterial pathogens causes bovine mastitis. A cross-sectional study was conducted from June 2022 to November 2022 to estimate the prevalence of mastitis in lactating cows, to assess the risk factors for bovine mastitis, and to isolate and identify coliform and gram-positive cocci bacteria involved in the mastitis cases from dairy farms in Dire Dawa, Eastern Ethiopia. Detection of mastitic animal was done based on physical examination of udders and CMT test. Bacterial culture and biochemical tests were employed to identify the target pathogens. A total of 366 dairy cows and 1,464 quarters were screened for mastitis. Overall prevalence of mastitis at cow and quarter levels were 24.04 and 13.5%%, respectively. Age, parity, cows udder position, history of mastitis, barn floor, milking sequences of clinically mastitic cows and leg and udder hygiene scores were found to be risk factors significantly ($P<0.05$) associated with mastitis. From the 191 mastitis-positive milk samples, 82.7% (158/191) were culture positive. Out of the isolates from clinical cases (n=59) and isolates from sub clinical cases (n=99), *Staphylococcus aureus* (22%) and *E. coli* (15.7%) were predominant isolate. The other bacterial isolate in order of abundance, Coagulase Negative Staphylococcus (10.5%), *Streptococcus agalactiae (6.8%), Streptococcus dysgalactiae (5.8%), Staphylococcus intermedius (4.7%), Staphylococcus hyicus (4.2%), Klebsiella pneumoniae (3.1%), Micrococci spp (2%), Streptococcus uberis (1.6%), Enterobacter aerogenes (1%), and Enterococci spp (0.5%).* The study showed that high parity number (OR = 19.5; p = 0.005), moderate parity (OR = 10.9; p = 0.022) and history of mastitis in preceding lactation (OR = 28.4; p = 0.001) were the major risk factors which are significantly associated with higher prevalence of *S. aureus*. History of mastitis in preceding lactation (OR = 3.7; p = 0.021) and very dirty (OR = 3.9; p = 0.005) udder and legs were the major risk factors which are significantly associated with higher prevalence of *E. coli*. Therefore, hygienic milking practice, adequate sanitation of the dairy environment, proper attention to the health of mammary glands and regular screening tests should get emphases as control strategies.

Keywords: Associated Risk Factor, CMT, Coliform, Isolation, Gram Positive Cocci, Mastitis, Prevalence

1. Introduction

Bovine mastitis is an inflammation of mammary glands caused by bacterial, viral or fungal pathogens, which is manifested by gross pathological changes of the udder as well as an elevated level of somatic cells in milk. It is a complex and multifactorial disease, resulting from the interaction of three major factors: the animal, pathogens, and environmental factors [1-3]. Mastitis is a global health problem of dairy animals and occurs in sporadic and epidemic forms resulting

into profound economic losses to dairy sector in developed and developing nation [4]. Furthermore, mastitis could be a danger to human health because milk from mastitic udder of animal is contaminated with bacteria, which could be potential source of infection to consumers [5], such as tuberculosis, streptococcal intoxication, colibacillosis, streptococcal sore throat, and brucellosis [1].

Mastitis is universally classified as clinical and subclinical based on the extent of inflammation [6]. Clinical mastitis is characterized mainly by appearances of changes in the milk such as flake and clots and presence of signs of inflammation on the mammary glands such as swelling, heat, pain, and edema as well as systemic signs on the animal including fever, rapid pulse, appetite loss, dehydration, and depression [7]. Subclinical mastitis is characterized by the absence of visible changes in the milk or udder, but with decrease milk production. Thus, its detection is not by clinical inspection but by determining the somatic cell count (SCC) in milk or by bacterial culture [8, 9].

Mastitis also classified as environmental and contagious based on bacterial agents involved in the disease process [1]. Environmental mastitis is caused by microorganism, which do not normally live on the skin or in the udder but enter the teat canal when the cow is exposed to a contaminated environment. The pathogens are normally found in feces, bedding materials, water supply, and feed, and transmitted by contact of the udder with those materials [1]. Bacteria such as coliforms Streptococcus dysagalactiae, Serratia, Pseudomonas and Streptococcus uberis [10, 11], cause environmental mastitis. Contagious mastitis are caused by contagious mastitis causing pathogens primarily exist in the infected mammary glands or on the cow's teat skin and transmit from infected to non-infected mammary glands during milking by milker's hand or milking machine liners [1]. Agents of contagious mastitis includes *Staphylococcus aureus, Streptococcus agalactiae, Corynebacterium bovis* and *Mycoplasma species* [12, 11].

Several species of bacteria, fungi, mycoplasmas, algae and virus [10. 8, 4] cause mastitis. Bacteria are the primary causes of mastitis, and more than 140 different pathogenic species of bacteria are implicated as the causes [13]. According to their prevalence and the severity of symptoms, the pathogens involved in the etiology of bovine mastitis can be classified into major and minor pathogens [14, 4]. Major pathogenic agent includes staphylococci (*Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus hyicus*), streptococci (*Streptococcus agalactiae, Streptococcus dysgalactiae* and *Streptococcus uberis,*), coliforms (mainly *Escherichia coli* and *Klebsiella pneumoniae*), *Enterococcus* spp., *Pseudomonas* spp., *Actinomyces pyogenes* and *Serratia* spp [15, 4, 16]. Minor pathogenic agent includes coagulase-negative Staphylococcus, bacilli spp, *Corynebacterium bovis, Mycoplasma,* and fungi (*Candida albicans, Cryptococcus neoformans, Geotrichum candidum,* and others) [1, 4, 16; 17] and viruses, such as bovine herpes viruses, cowpox virus, pseudo cowpox, vesicular stomatitis, foot and mouth disease viruses [18].

Mastitis accounts for the largest economic losses on dairy farms in many countries in the world, including the USA, United Kingdom, Europe, Australia and South Africa [19] as well as in Ethiopia [20, 21]. Clinical and sub clinical mastitis causes economic losses due to production losses, culling, changes in product quality and the risk of other diseases. Mastitis has been known to cause a great deal of loss or reduction of productivity, to influence the quality and quantity of milk yield, and cause culling of animals at an unacceptable age [22, 23]. Most estimates have shown a 30% reduction in productivity per affected quarter and 15% reduction in production cow or lactation, this making the disease one of the most costly and serious problem affecting the dairy industry worldwide [24]. Morin *et al*. [25] estimated the economic loss from mastitis on dairy farms of United state approximately US$ 200 per cow/year, which causes an annual loss of 2 billion dollars. Mungube [26] estimated the economic loss from mastitis in the periurban areas of Addis Ababa as 210.8 ETB/cow/lactation from which milk production loss contributed to 38.4%. In addition, another study indicated the average loss of 17.2% in milk production due to mastitis in Ethiopia [27].

Ethiopia has a large livestock resource in Africa with a total of 57.8 million cattle population of which 7.2 million are mainly kept for the processing of milk [28]. Milk produced from these animals provides an important dietary source for the majority of rural as well as a considerable number of the urban and peri-urban population. However, milk production often does not satisfy the country's requirements due to a multitude of factors, out of which disease of the mammary glands known as mastitis is among the various factors contributing to reduced milk production [29, 21]. The prevalence of bovine mastitis has been reported from several parts of our country. Accordingly, the reported prevalence were, 99.9% in selected areas of southern Ethiopia [30]; 51.1% in Eastern part of Amhara region [31]. Ejeta *et al*. [32] in and Around Ambo (42%); Fesseha *et al*. [33] in Modjo (73.3%), Etifu and Tilahun [34] in Mid Rift valley (73%), Abebe *et al*. [35] in Southern Ethiopia (54.2%); Tesfaye and Abera [36] in and around Haramaya (49.2%), Jafer *et al*. [37] in Dire Dawa (39.2%).

Dire Dawa city is one of the densely populated and fastest growing cities in our country. The increasing population density is leading to increased demand for milk consumption. Therefore, many farmer and business owner plant dairy farm as one investment with aims of high milk production. In this specified area, the mastitis is reported as one of the challenges to considerable number of dairy producers [37-39]. Even though there are many published paper on prevalence and risk factor of mastitis, there are few reports stating in relation to isolation of coliforms and gram-positive cocci bacteria bacterial species that are commonly involved in clinical and sub clinical mastitis. On the other hand, most of the previous studies in Ethiopia were concentrated on the investigation of the prevalence and few risk factors for mastitis at cow level and no or little effort has been made to assess the prevalence, management and hygienic practices at herd/farm-level and

also recent data is lacking regarding which bacterial pathogens prevalent in the area. Thus, filling the mentioned gap has great importance for designing feasible prevention and control strategy in the area as well as other farms with similar settings.

Therefore, this research is aimed with the following objectives:

General objective of study

To study the epidemiology of coliform and gram-positive cocci bacteria among cases of bovine mastitis in selected dairy farms of Dire Dawa.

Specific objective of study

1) To determine the prevalence of clinical and sub clinical mastitis.
2) To isolate and identify coliform (*Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter aerogenes)*, Staphylococcus species (*Staphylococcus aurues, Staphylococcus intermedius and Staphylococcus hyicus*), Streptococcus species (*Streptococcus agalactiae, Streptococcus dysagalactiae* and *Streptococcus ubris*) and other gram-positive cocci bacteria from mastitic milk samples.
3) To assess the risk factors associated with the occurrence of different mastitis pathogens.

2. Methods and Methods

2.1. Study Area

The present study was conducted in selected dairy farms of Dire Dawa town from June 2022 to November 2022. Dire Dawa is situated in the Eastern part of Ethiopia at about 515km of Addis Ababa. It is located the approximately between Latitude 9°27' and 9°49' N and Longitude 41°38' and 42°19' E. The entire territory of Dire Dawa administrative council rests on an elevation ranging between 950m. a. s. l. in the North East to 2260m. a. s. l. in the south West. Moreover, Dire Dawa administrative council shares boundary to the south, South East and South West with Eastern Hararghe Zone of the Oromia regional state and the North, North East and West with Shinile Zone of Somali regional state. The Dire Dawa administrative council have a bi modal type of rainfall and characterized by small rainy season from February to May and heavy rainy season from July to September. The dry season extends from October to January. The mean annual rainfall of Dire Dawa administrative council varies from 550mm in the lowland northern part to above 850mm in the southern mountain. The average annual rainfall of the study area was 604mm. The monthly mean minimum and maximum temperature of Dire Dawa administrative council ranges from 14.5°C to 34.6°C respectively. Using the1500m contour as a line of separation, Dire Dawa administrative council had two agro-ecological zones; the *kola* (below 1500m) and *Woinadega* (above 1500m) have been recognized in Dire Dawa administrative council [40]. There are about 180,796 head of cattle, 184,507 goats, 23,723 sheep, 10,899 equines, 91,894 camels and 175,305 poultry in Dire Dawa administrative council [28]. All of these livestock species are reared by small scale, medium and large-scale farms under extensive and intensive production system.

2.2. Study Population

The study animals were all lactating dairy cows found in the randomly selected cow's population from conveniently selected 24 dairy farms in Dire Dawa, Eastern Ethiopia. The farms were categorized into SSDP (Small-scale dairy production), MSDP (Medium scale dairy production) and LSDP (Large-scale dairy production) based on their herd size that is less than or equal to five, 6 up to 70 and above 70 respectively [41]. The animals were kept indoors and are supplemented with concentrates, by products of beer, molasses and hay. Most of the farms were intensive production in which dairy animals are kept indoors at zero grazing.

2.3. Study Design

A cross-sectional study was employed from June 2022 to November 2022 in Dire Dawa town to determine prevalence bovine mastitis, assessed associative risk factors and to isolate and identify gram-positive cocci and coliform bacteria.

2.4. Study Methodology

2.4.1. Questionnaire Survey

During farm visits, semi-structured questionnaire was used to collect data on herd and individual cow variables thought to influence the occurrence of mastitis. Data was collected from farm owners/attendants through a face-to-face interview. Some of the information were recorded by direct observation of the milking and husbandry practices. The herd level variables that were recorded includes herd size, floor type (concrete or soil), bedding (yes or no), udder washing practices (whole udder or teats only), housing (stall barn or group barn), use of towel for drying (yes or no), whether mastitis cows milked last or not. Cow level variables that were recorded included age, parity, stage of lactation, udder position (normal or pendulous), cow leg and udder dirtiness (clean, slightly dirty, moderately dirty or very dirty), and previous history of mastitis.

The study cows were categorized into the different age groups young (less than five years of age), Adult (between five to eight years of age) and Old (above eight years of age) groups of the cows. Age of cows were determined by observing their dentition characteristics [43] and by observing birth records. The study animals were categorized into the different parity groups according to Bedacha and Mengistu [41] into few (1-3 calves), moderate (3-6 calves) and many (6 and above calves). Milking hygiene practice was grouped into good (If there is a practice of washing and drying udder with separate towels, milking healthy and young cows first) and poor (If washing and drying of udder with a separate towel and milking with order is not practiced). Lactation stage of the cow was also categorized into early stage lactation (1 -3 months), mid lactation (4–7 months) and late lactation (above 7 months) according to [35]. The udder and leg hygiene of

each cow was assessed and scored based on a four-point scale 1–4 ('1' was referred to no contamination of the skin of the rear of the udder or the hind limb between the hock and coronary band; '2' slightly dirty (2–10% of the area covered in dirt; '3' moderately dirty (10–30% of the area covered in dirt); and '4' caked-on dirt (>30% of these areas completely covered in dirt) as described by Schreiner and Ruegg [44].

2.4.2. Physical Examination of the Udder

Clinical mastitis was diagnosed at the quarter level based on visible and palpable signs like hard and swollen quarter, pain (kicking up on touching the udder), size and consistency of mammary quarters were inspected for the presence of any anatomical malformation, such as disproportional symmetry, swelling, firmness, blindness and heat [1].

2.4.3. California Mastitis Test

The California mastitis test was conducted to diagnose the presence of subclinical mastitis and it was carried out according to procedures given by Quinn *et al*. [4]. The udder of the cows to be tested was cleaned with water and antiseptics and dried with clean towel. Then the first three stream of milk were discarded from each quarter. Appropriate amount of milk sample were poured into four shallow cups in the CMT paddle from each quarter paddle, an equal amount of the CMT reagent (4% sodium hydroxide) was added to each cups, 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 test result of CMT 1 to 3, were classified as evidence of subclinical mastitis.

2.4.4. Milk Sample Collection and Transportation

Milk was collected from both clinical and subclinical mastitis cases using standard milk sampling techniques adopted by the National Mastitis Council [45]. First, after the hands were cleaned by soap and clean water the lactating cow udder and teat orifice has been completely washed by clean water and dried by dry clean towel before collecting milk samples. Then, the teats were further scrubbed with cotton, soaked in 70% ethyl alcohol to prevent recontamination. Teats on the far side of the udder were first scrubbed with alcohol and sampled, and then those on the near side were sampled later. Approximately 5–10 ml of milk was collected from each teat aseptically in separate sterile test tube after first three streams of milk were discarded. Finally, all milk samples were labeled and transported in an icebox to the Microbiology Laboratory of Dire Dawa regional Veterinary Laboratory, where they were stored at 4°C for a maximum of 24 hrs until inoculation on a standard bacteriological media was done.

2.4.5. Isolation and Identification of Target Bacteria

Bacteriological examination of milk samples was done in accordance with the procedures used by. We present a bacteriological analysis of milk samples from both clinical and subclinical quarters using the standard bacteriological protocols [46]. Milk samples obtained from each teat quarter were individually cultured using 7% defibrinated bovine blood on MacConkey agar and blood agar bases and incubated aerobically at 37°C for 24–48 hours. Then, plate growth, morphology, and hemolysis pattern on the blood agar base were subsequently studied. Subcultures were made for the pure identification of isolates.

The growth of bacteria on mannitol salt agar and purple agar was used to identify Staphylococci species. The fermentation of mannitol by *S. aureus* causes yellow discoloration of the medium. Colonies that show a weak or delayed yellow color on Mannitol Salt Agar (MSA) after 24 hrs of incubation were considered as *S. intermedius* and colonies that failed to produce any change in the medium were determined as *S. hyicus* and CNS [46]. On the other hand, colonies that were grown on the MSA plate were sub-cultured on nutrient medium broth and incubated at 37°C for 24 hrs. Then, 0.5 mL of rabbit plasma and a drop of the 24 hrs old colonies taken from nutrient broth (NB) were mixed and incubated for 4–24 hrs at 37°C. The clotting of the suspension was evaluated at 30 minutes intervals for the first 4 hrs of the test and then after 24 hrs of incubation. The reaction was considered as coagulase-positive if any degree of clotting was visible. The detection of Streptococci species was performed on Edwards's media according to their growth characteristics. Different biochemical tests, such as Tube coagulase test, catalase test, esculin hydrolysis test, indole production, methyl red test, Voges-Proskauer reaction, urease production, citrate utilization, and sugar fermentation, were used to identify the Staphylococci and Streptococci species [46].

In addition, pink-colored presumptive *E. coli* colonies were sub-cultured onto Eosin Methylene Blue (EMB). Colonies with a metallic green sheen on EMB were later characterized microscopically using Gram's stain. Presumed *E. coli* colonies were then transferred onto nutrient agar for further identification using biochemical tests. Oxidase reaction, Catalase testing, Triple Sugar Iron (TSI), "IMViC" (indole, methyl red, Voges-Proskauer, and citrate) test, and motility test have been used to identify the *E. coli* species [46].

2.5. Sampling Method and Sample Size Determination

In this research, initially Dire Dawa town were purposively selected from the Eastern Ethiopia towns based relatively high number of dairy farms and the increasing demand for milk due to urbanization in this town. A total of 24 dairy farms were first selected using convenience sampling techniques from total 31 commercially register dairy farms in the study area on the basis of accessibility and willingness of the farm owners to participate in the study. Then, the lactating dairy cows were selected from 24 dairy farms using a simple random sampling technique. The total number of lactating dairy cows was determined based on the number of the cattle population in each farm. The desire sample size for the study was calculated based on the formula developed by Thrusfield [42] for simple random sampling. The number of the sample was determined using 95% level of confidence, 0.05-desired absolute precision and by taking 39.2% of expected prevalence that can be taken from previous study conducted by Jafer *et al*. [37].

$$n = \frac{1.96^2 * P_{exp}(1-P_{exp})}{d^2}$$

Where n = required sample size P_{exp} =Expected prevalence d^2 = Desired absolute precision (5%) Based on this formula, the total numbers of respondents to be included in the questionnaire survey were 366.

2.6. Data Management and Analysis

Collected data were entered into a Microsoft Excel spreadsheet and subjected for data filter before being analyzed then STATA 14 software was used to analyzed data. Descriptive statistical analysis was used to summarize and present collected data. The prevalence of mastitis was calculated as the number of lactating cows tested positive by CMT test and animals showing symptoms of clinical mastitis, divided by the total number of tested or clinically examined animals. The existence of association between the risk factors (age, parity, breed, lactation stage, leg and udder dirtiness, previous history of mastitis floor type, housing system and milking hygiene) and mastitis was assessed using the Pearson Chi-square ($\chi 2$) test. Besides, the degree of association between the risk factors and occurrence of mastitis were analyzed first with univariate logistic regression. The results were considered significant at $P<0.05$.

3. Results

3.1. Prevalence of Bovine Mastitis at Cow and Quarter Level

From the 366 examined cows, the overall prevalence of mastitis at the cow level was found to be 24.04% (88/366). The prevalence of clinical and subclinical mastitis were 6.01%, and 18.03%, respectively (Table 1). From total 1,464 quarters examined, 3.28% teats were found blind. From the 1,416 functional teats examined, the overall prevalence of mastitis was found 13.5% (*n* = 191), of which 4.3% (*n* = 61) quarters showed clinical mastitis. From those quarters screened by CMT, 9.18% (*n* = 130) showed evidence of subclinical mastitis (Table 2). Out of the total quarter with sub clinical mastitis, 22.3% (29/130), 46.2% (60/130) and 31.5% (41/130) were showed + (weakly positive), ++ (distinctive positive), and +++ (strongly positive) CMT positives score respectively. The herd level prevalence was found to be 100% (24/24) herd level prevalence illustrated on (Table 3).

Table 1. *The prevalence of clinical and subclinical mastitis at cow level.*

Types of mastitis	No of Examined	No of Affected	Prevalence (%) 95%CI
Clinical	366	22	6.01 [3.6 – 8.5]
Subclinical	366	66	18.03 [14 – 22]
Total	366	88	24.04 [19.6 – 28.4]

Table 2. *Prevalence of clinical mastitis, subclinical mastitis and blind teats across quarters.*

Quarter	Blind teats	Prevalence of SCM	Prevalence of CM	Mastitis prevalence
RR	17 (4.6%)	38 (10.88%)	15 (4.3%)	53 (15.2%)
LR	8 (2.2%)	39 (10.89%)	19 (5.3%)	58 (16.2%)
LF	11 (3%)	29 (8.17%)	15 (4.2%)	44 (12.4%)
RF	12 (3.3%)	24 (6.78%)	12 (3.4%)	36 (10.2%)
Total	48 (3.3%)	130 (9.18%)	61 (4.3%)	191 (13.5%)

Table 3. *The Prevalence of clinical and subclinical mastitis at herd level.*

Farm name	Examined	Clinical	Subclinical	Total
1	18	1 (5.6%)	5 (27.8%)	6 (33.3%)
2	29	0	6 (20.7%)	6 (20.7%)
3	27	2 (7.4%)	5 (18.5%)	7 (25.9%)
4	15	2 (13.3%)	2 (13.3%)	4 (26.7%)
5	12	2 (16.7%)	2 (16.7%)	4 (33.3%)
6	10	0	1 (10%)	1 (10%)
7	21	2 (9.5%)	2 (9.5%)	4 (19%)
8	18	1 (5.6%)	6 (33.3%)	7 (38.9%)
9	7	0	1 (14.3%)	1 (14.3%)
10	14	0	4 (28.6%)	4 (28.6%)
11	11	1 (9%)	1 (9%)	2 (18.2%)
12	10	1 (10%)	3 (30%)	4 (40%)
13	9	1 (11.1%)	1 (11.1%)	2 (22.2%)
14	7	0	2 (28.6%)	2 (28.6%)
15	11	1 (9%)	0	1 (9%)
16	18	2 (11.1%)	4 (22.2%)	6 (33.3%)
17	18	2 (11.1%)	4 (22.2%)	6 (33.3%)
18	11	0	2 (18.2%)	2 (18.2%)
19	10	0	1 (10%)	1 (10%)
20	31	1 (3.2%)	3 (9.7%)	4 (12.9%)
21	10	0	2 (20%)	2 (20%)
22	18	2 (11.1%)	4 (22.2%)	6 (33.3%)
23	20	1 (5%)	3 (15%)	4 (20%)
24	11	0	2 (18.2%)	2 (18.2%)
Total	366	22 (6.01%)	66 (18.03%)	88 (24.04%)

3.2. Association of Risk Factor with the Occurrence of Bovine Mastitis

The result of analysis of different intrinsic and extrinsic risk factors are summarized in tables 6 and 7. The occurrence of mastitis was 3.99 times higher (OR=3.99; 95% CI=2.4-6.59; *P*=0.0001) in pendulous udder than normal one. Also, cows with many calving (> 6 calves) and moderate calving (3–6 calves) had 3.42 (OR= 3.42; 95% CI: 1.8-6.55) and 2.26 (OR= 2.26; 95% CI: 1.27-4) times, respectively the chance to encounter mastitis as compared to cows with few calving (1–3 calves) and the association was significant (p< 0.001). Moreover, the odds of cows being infected by mastitis was 2.75 times higher in cows aged >8 years (OR= 2.75, 95% CI: 1.4–5.16) and 1.51 times higher in cows aged between five to eight years (OR= 1.51, 95% CI: 0.77–2.95) than aged <5 years

and there was statistically significant difference (P= 0.005) occurrence of mastitis among the in the age groups (Table 4).

Table 4. *Association between intrinsic risk factors and prevalence of mastitis.*

Intrinsic Risk Factor	Categories	No of Examined	Prevalence (%)	OR 95% CI	X^2	P value
Udder Position	Normal	229	14.41	Ref	31	0.000
	Pendulous	137	40.15	3.99 (2.4-6.59)		
Breed	Local	125	20.80	Ref	1.09	0.296
	Cross	241	25.73	1.31 (.78-2.22)		
Age	Young	103	15.53	Ref	10.73	0.005
	Adult	138	21.74	1.51 (.77– 2.95)		
	Old	125	33.60	2.75 (1.4 –5.16)		
Parity	Few	157	14.65	Ref	15.4	0.000
	Moderate	136	27.94	2.26 (1.27-4)		
	Many	73	36.99	3.42 (1.8-6.55)		
Stage of Lactation	Late	112	18.64	Ref	3.7	0.154
	Mid	87	20.69	1.07 (.53-2.1)		
	Early	167	28.74	1.7 (.93-2.93)		
History of Mastitis	No	231	14.29	Ref	32.65	0.000
	Yes	135	40.74	4.1 (2.5-6.8)		

Among environmental factors, the analysis showed that milking mastitic cows last, floor type, and milking practices were found to be significantly ($p < 0.05$) associated with the occurrence of mastitis. Thus, the prevalence of mastitis was 2.16 times higher (OR=2.16; 95% CI=1.3-3.5; P=0.002) in animals managed under farms that did not practice milking of mastic cows at last. (Table 5).

Table 5. *Association between extrinsic risk factors and prevalence of mastitis.*

Extrinsic Risk Factors	No Examined	No Affected	Prevalence (%)	OR (95% CI)	X^2	P value
Leg and Udder Dirtiness					25.22	0.000
Clean	85	9	10.59	Ref		
Slight dirty	155	33	21.29	2.28 (1.04- 5)		
Moderate dirty	70	20	28.57	3.38 (1.42- 8)		
Very dirty	56	26	46.43	7.32 (3.1- 17.4)		
Housing System					0.2	
Group barn	272	67	24.63	Ref		0.652
Stall barn	94	21	22.34	0.88 (.5 – 1.54)		
Bedding					0.12	
Yes	282	69	24.47	Ref		0.728
No	84	19	22.62	0.9 (.50 - 1.6)		
Floor types					3.96	
Concretes	154	29	18.83	Ref		0.047
Soil	212	59	27.83	1.7 (1. -2.7)		
Milking Mastitic Cow Last					9.9	
Yes	219	40	18.26	Ref		0.002
No	147	48	32.65	2.16 (1.3 -3.5)		

3.3. Prevalence of Bacteria Species in Mastitic Milk

Out of the 191 milk samples (61 from clinical and 130 from subclinical mastitic quarter) subjected for bacterial culture, evidence of bacterial growth on cultural media was observed only in 82.7% (158/191) quarter. Based on sample type, frequency of bacterial culture was 76.2% (99/130) in subclinical mastitis infected quarter, while it was 59/61 (96.7%) in quarters with clinical mastitis (Table 6). The isolation frequency of *S. aureus*, *S. intermedius*, *S. hyicus*, Coagulase Negative Staphylococcus (CNS), *Str. dysgalactiae*, *Str. agalactiae*, *Str. ubris, Micrococci spp, Enterococci spp, E. coli, Klebsiella pneumoniae* and *Enterobacter aerogenes*is presented in (Table 7).

Table 6. *Frequency of cultural growth from culture of mastitic milk sample across four quarter.*

Quarter	Subclinical mastitis		Clinical mastitis		Total growth %
	No of culture	Growth on culture	No culture	Growth on culture	
RR	38	31 (81.6%)	15	15 (100%)	46 (86.8%)
LR	39	30 (76.9%)	19	19 (100%)	49 (84.5%)
LF	29	21 (72.4%)	15	14 (93.3%)	35 (79.5%)
RF	24	17 (70.8%)	12	11 (91.7%)	28 (77.8%)
Total	130	99 (76.2%)	61	59 (96.7%)	158 (82.7%)

The overall isolation proportion of each bacteria in mastitic milk were 22, 15.7, 10.5, 6.8, 5.8, 4.7, 4.2, 3.1, 2, 1.6, 1 and

0.5% for *S. aureus, E. coli,* Coagulase Negative Staphylococcus (CNS), *Streptococcus agalactiae Streptococcus dysagalactiae, Staphylococcus intermedius, Staphylococcus hyicus, Klebsiella pneumoniae, Micrococci spp, Streptococcus ubris, Enterobacter aerogenes* and *Enterococci spp,* respectively. *Staphylococcus aureus* was the dominant isolate from clinical and subclinical cases with frequencies of 29.5% and 18.5%, respectively. In addition, *E. coli* was the second predominant isolate from clinical and subclinical cases with frequencies of 19.7 and 13.8%, respectively.

Table 7. *Prevalence of bacteria isolated from clinical and subclinical mastitis.*

Bacteria	No of isolate from mastitis		
	CM (*n*=61)	SCM (*n*=130)	Total (*n*=191)
Staphylococcus aureus	18 (29.5%)	24 (18.5%)	42 (22%)
E. coli	12 (19.7%)	18 (13.8%)	30 (15.7%)
Coagulase negative Staphylococcus *(*CNS)	9 (14.8%)	11 (8.5%)	20 (10.5%)
Streptococcus agalactiae	3 (4.9%)	10 (7.7%)	13 (6.8%)
Streptococcus dysagalactiae	2 (3.3%)	9 (6.9%)	11 (5.8%)
Staphylococcus intermedius	4 (6.6%)	5 (3.8%)	9 (4.7%)
Staphylococcus hyicus	3 (4.9%)	5 (3.8%)	8 (4.2%)
Klebsiella pneumoniae	2 (3.3%)	4 (3%)	6 (3.1%)
Micrococci spp	2 (3.3%)	2 (1.5%)	4 (2%)
Streptococcus ubris	-	3 (2.3%)	3 (1.6%)
Enterobacter aerogenes	-	2 (1.5%)	2 (1%)
Enterococci spp	-	1 (0.8%)	1 (0.5%)
Other bacteria	4 (6.6%)	5 (3.8%)	9 (4.7%)
Total	59 (96.7%)	99 (76.2%)	158 (82.7%)

3.4. Association of Predominant Bacterial Species with Major Risk Factors

The current investigation revealed that the prevalence of *S. aureus* was significantly higher in quarters from old cows than young cow (P=0.001). The likelihood of the occurrence of *S. aureus* in adult cows is 5.9 and 1.9 times lower than in old cow and young cow, respectively. Parity of cow was significantly associated with the occurrence of *S. aureus* in mastitic milk samples, in that it was 2.6%, 22.2% and 33.9% in cows with few, moderate and many calving, respectively. In addition, previous history of mastitis was significantly associated with the occurrence of *S. aureus* (p=0.001), in that milk samples from cows with previous history of mastitis had 28.4 times the chance of harboring *S. aureus* (OR=28.4; 95%CI=3.8-112; *P*=0.001). However, leg and udder dirtiness of cow and breed of cows were found insignificantly associated (p > 0.05) with the occurrence of S. *aureus* in quarters (Table 8).

Table 8. *Association of major risk factors with prevalence of S. aureus.*

Risk factors	No sample	No positive	Prevalence (%)	OR 95% CI	P value
Age					
Adult	60	5	8.3	Ref	
Young	27	4	14.8	1.9 (0.5-7.8)	0.364
Old	104	33	31.7	5.9 (1.9-14)	0.001
Parity					
Few	39	1	2.6	Ref	
Moderate	90	20	22.2	10.9 (1.4-84)	0.022
Many	62	21	33.9	19.5 (2.5-151)	0.005
leg and udder dirtiness					
Clean	15	1	6.7	Ref	
Slight dirty	64	8	12.5	2 (0.2-17.3)	0.529
Moderate dirty	62	21	33.9	7.2 (0.9-58.3)	0.065
Very dirty	50	12	24	4.4 (0.5-37.2)	0.171
History of Mastitis					
No	62	1	1.6	Ref	
Yes	129	41	31.8	28.4 (3.8-112)	0.001
Stage of Lactation					
Mid	40	7	17.5	Ref	
Late	45	15	33.3	2.4 (0.4-18.5)	0.263
Early	106	20	18.89	1.2 (0.6-2.4)	0.142

This study revealed that leg and udder dirtiness of cow was significantly associated with the occurrence of *E. coli* in mastitic milk samples (p=0.005). Thus, the likelihood of the occurrence of *E. coli* in cow, which had very dirty leg and udder, was four times higher than in cow had moderate dirty leg and udder. Moreover, milk samples from cows with history of mastitis had 3.7 times (OR=3.7; 95%CI=1.2-11; *P*=0.021) the chance of harboring *E. coli* (Table 9).

Table 9. *Association of Major Risk Factors with Prevalence of E. coli.*

Risk factors	No sample	No positive	Prevalence (%)	OR 95% CI	P value
Age					
Young	27	2	7.4	Ref	
Adult	60	6	10	1.4 (0.3-7.4)	0.700
Old	104	22	21.2	3.4 (0.7-15.3)	0.118
Parity					
Few	39	-	-	-	
Many	62	12	19.4	Ref	
Moderate	90	18	20	1.04 (0.5-2.4)	0.922
leg and udder dirtiness					
Clean	15	-	-	-	
Slight dirty	64	-	-	-	
Moderate dirty	62	9	14.5	Ref	
Very dirty	50	21	42	3.9 (0.5-12.3)	0.005
History of Mastitis					
No	62	4	6.5	Ref	
Yes	129	26	20.2	3.7 (1.2-11)	0.021
Stage of Lactation					
Mid	40	4	10	Ref	
Late	45	10	22.2	2.6 (1.8-5.4)	0.075
Early	106	17	16	1.6 (0.7-3.8)	0.296

4. Discussion

In the present study, the overall prevalence of mastitis was found 24.03% (95% CI: 19.6 – 28.4) this finding is in line with previous authors' report from Ethiopia such as Yohannes and Alemu [47], Zerfu *et al*. [48], Demelash *et al*. [49] and Girma *et al*. [50] who reported 24.9% (in Wolayta Soddo); 25% (in Boke district); 23% (in southern Ethiopia) and 23.18% (in Doba district), respectively. However, it was higher than reported by Belay *et al*. [51] in Gamo Zone of Southern Ethiopia; Kasech *et al*. [52] in Tullo district of West Hararegheː Tesfaye [53] in Debrezeit; and Abraham and Zeleke [54] in and around Wolaita Sodo who showed an prevalence of 17.1%; 16.1%; 6%; and 5% respectively. On the other hand higher prevalence than the present finding was reported by Ejeta *et al*. [32] in and Around Ambo (42%); Fesseha *et al*. [33] in Modjo (73.3%), Etifu and Tilahun [34] in Mid Rift valley (73%), Abebe *et al*. [35] in Southern Ethiopia (54.2%); Tesfaye and Abera [36] in and around Haramaya (49.2%), Biniam *et al*. [38] in Dire Dawa town (53.25%). Variations in prevalence might be due to the complex nature of mastitis and its interactions with several factors, such as management and husbandry practices, environmental conditions and animal-level factors [55].

The present study revealed that 100% of the herds observed had at least one cow suffering from mastitis. The herd level prevalence report of this investigation is much higher than reports of Abebe *et al*. [56] in dairy herds at Hawassa (74.7%); Fesseha *et al*. [33] in dairy farms of Modjo (74.4%); and Mdegela *et al*. [57] in smallholder dairy farms in Tanzania (21.7%). Perhaps this high herd level prevalence of mastitis could either be due to lack of implementation of regular mastitis prevention or control strategies. The fact that none of the dairy farms implementing routine mastitis prevention practices such as post-milking teat disinfection, wearing of gloves during milking, dry cow therapy, culling of chronically infected and old cows and none of the farmers were doing CMT or other tests routinely to screen their cows for subclinical mastitis.

In current study the prevalence of subclinical mastitis (18.03%, 95% CI: 14–22) was higher than that of clinical mastitis (6.01%, 95% CI: 3.6–8.5). These findings of higher prevalence of subclinical mastitis as compared to clinical mastitis prevalence is in agreement with other previous findings of Abebe *et al*. [35], Christine *et al*. [58], Dabele *et al*. [59], Amin *et al*. [60] and Zerfu *et al*. [48] who found higher prevalence of subclinical mastitis as compared to clinical mastitis prevalence. The subclinical forms of mastitis more prevalent, long duration and of high economic consequence than clinical forms of mastitis and usually precedes the clinical form [61]. As a result of the defense mechanism of the udder, which tends to reduce the severity of the disease, the subclinical form of mastitis has also been suggested to be higher than that of clinical mastitis [6].

The cow level prevalence of clinical mastitis reported in the present study (6.01%) is in agreement with the finding of Abebe *et al*. [35], who reported the prevalence of clinical mastitis to be 6% in Southern Ethiopia. Moreover, Lakew *et al*. [62] reported 6.77% in and around Haramaya district, Eastern Ethiopia. But the current clinical mastitis prevalence report is much lower than reported by Fesseha *et al*. [33] from Modjo town of Central Ethiopia (28.9%), where as it is far higher than reported by Zerfu *et al*. [48] from Boke district West Hararghe Zone and Belay *et al*. [51] from Gamo Zone of Southern Ethiopia at proportions of 2 and 1.9%, respectively. Risk factors which influence the occurrence of clinical mastitis were outlined as animal, pathogen, and environmental risk factors, which could contribute in the discrepancies of mastitis prevalence [1].

The cow level prevalence of sub clinical mastitis reported in the present study (18.03%) is in line with the finding of

Yohannes and Alemu [47] who found 19.6% in and around Wolayta Soddo, while the current finding is lower than reported by Fesseha *et al*. [33] from Modjo Town of Central Ethiopia (71.02%) and by Kitila *et al*. [63] from West Wollega (22.7%). However, lower prevalence (9.7%) was reported by Sefinew *et al.* [64] from Gondar. This could be attributed to the invisible and silent nature of subclinical mastitis, which is usually given little attention by farms owners when it comes to treatment unlike clinical mastitis towards which treatment and control efforts are concentrated [65].

The current study revealed that 13.5% of quarters were affected by mastitis, which is in line with the report of Bitew *et al*. [66] (12.3%) in and around Bahir Dar town of the country. But Dabele *et al*. [59] reported lower prevalence (8.3%) in districts of West Shewa Zone. Likewise, Belay *et al*. [51] reported a prevalence of 7.6% in Gamo Zone of Southern Ethiopia, Kumbe *et al*. [67] reported 21.48% in Borana Zone, and Lakew *et al*. [62] reported 29.04% in and around Haramaya district, Eastern Ethiopia. From this study, it was observed that mastitis was higher in the left rear (16.2%) and right rear (15.2%) quarters when compared to other quarters. This finding is in line with other reports of Belay *et al*. [51]. The highest infection level in the rear quarters might be because of the hindquarters' greater production capacity and higher chance of environmental and fecal contamination, owing to their anatomical location [68].

Based on present study, the age cows was one of predisposing factor for mastitis occurrence (p = 0.005), with higher proportion to be recorded in old cows (above eight years old) (33.60%) than adults (between five and eight years of age) (21.74%) and young cows (less than five years old) (15.53%). This finding is in line with findings of previous research worked by [33, 34, 35, 69]. High prevalence mastitis in older cows was because of their largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agent in the cows' udder [1]. Even though statistically not significant association between breed of cow and mastitis, the prevalence of mastitis was higher in cross breed of cows (25.73%) than the local breed of cows (20.80%).

Parity was found a significant influence on the prevalence of mastitis. Highest prevalence of mastitis recorded in cows having greater than six calves (36.99%) followed by cows having between three to six calves (27.94%) and cows having less than three calves (14.95%). Hence, the occurrence of bovine mastitis in cows having greater than six calves were 3.34 times higher than those in cows having less than three calves. This report is in accordance with previous reports of Abebe *et al*. [35], Lakew *et al*. [62], Belina *et al*. [70] and Girma *et al*. [50] who reported cows having greater than six calves were more prone to mastitis than cows having less than three calves. This might be due to repeated parturition increase the patency of the teats and decreases the local defense mechanisms. In addition, repeated parturition also exposes cows to environmental and contagious bacteria. Besides, multiple parturition stresses cows and ultimately down regulates their immunity [1].

This study found that cows with more pendulous udders position were more prone to bovine mastitis than cows having normal udder position. The likelihood of getting mastitis was 3.99 times higher in cows with pendulous udders than cows with normal udder position. This finding is in line with previous reports of Belay *et al*. [51], Firaol *et al*. [71] and Kumbe *et al*. [67], who reported cows with pendulous udders to have a higher incidence of mastitis than cows with non-pendulous udders. This could be due to pendulous udders open the teats and udders to injury, and microbes readily adhere to the teats and gain entry to gland tissue [72].

In case of a study there were not significant difference was found between cows stage of lactation to the mastitis occurrence (p = 0.154). This result is in agreement with the previous findings by Yohannes and Alemu [47], Demissie *et al*. [69] and Yenew and Addis [73], which indicated higher prevalence of bovine mastitis in cows of early stage of lactation. Perhaps this could be linked to the fact that diapedesis of neutrophils into the mammary gland take longer time in recently calved cows [1], and increased oxidative stress and reduced antioxidant defense mechanisms during early lactation [74]. Moreover, absence of dry cow therapy regime could possibly be among the major factors contributing to higher prevalence at early lactation.

The prevalence of mastitis in cows, which had history of mastitis, and cows, which not have history of mastitis, was 40.74% and 14.29% respectively. In their report Abebe *et al*. [35], Fesseha *et al*. [33] and Abebe *et al*. [56], showed that 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 [68].

The odds of finding a cow with mastitis increased as the degree of cows leg and udder dirtiness increased. It was noted that the likelihood of mastitis was 7.32, 3.38 and 2.28 times higher in cows with very dirty, moderately dirty and slightly udder and legs dirtiness as compared to those with relatively clean udder and legs, respectively. It is obvious that the dirtiness of udder and hind legs is the result of poor hygiene of the cow's environment and facilities in the cows' barn. As stated by Rajabi *et al*. [75], poor cow hygiene can contribute to presence of mastitis pathogens on teat ends and increasing the rate of new infections. Similar to the current finding, other researchers have also reported a significant association between mastitis prevalence and poor udder and leg hygiene [76, 56].

Prevalence of mastitis was significantly associated with milking hygienic practice. Cows in herd that had poor milking hygiene standard were significantly more likely to have mastitis than those with good milking hygiene practices. This finding is in agreement with previous studies in the country [77, 78, 33]. This might be due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths, which could serve as a vehicle to spread especially for contagious mastitis.

This study showed that cows in herds that did not practice

milking mastitic cows last were significantly more likely to have mastitis than those that did milking mastitic cow last. This report is in agreement with the reports by Abebe *et al* [56], Ejeta *et al*. [32] they reported that failure to milk mastitic cows last increased the spread of mastitis in farms from one cow to another during milking. These findings may explain the reason for high farm-level prevalence in this study so farm owner and attendant need to be educated on the importance of knowing the cow's udder health status and encouraged to milk mastitic cows last to prevent the spread of mastitis.

The study also showed that there was statistically significant association (p = 0.047) of mastitis floor types, in that cow living in non-concrete house had higher (27.83%) mastitis prevalence than those living in barn with concrete floor (18.83%). This finding is in agreement with previous finding of Lakew *et al*. [62] and Kassa *et al*. [79] who found the cows, which lived in soil barn floor, were more prone bovine mastitis than cows which lived concrete barn floor.

Bacteriological processing of milk samples from 191 infected quarter showed that the growth of various groups of bacteria in 158 (82.7%) samples. This finding is comparable to Yenew and Addis [73] in Wallo (85.7%), but higher than the finding of Kumbe *et al*. [67] in Borna Zone (46.97%). In addition, it was lower than the finding of Abebe *et al*. [56] who observed 98.8% (170/172) bacterial growth from 18 milk samples from 18 clinical mastitis infected cow and 154 samples from 313 sub clinically infected cows and cultured only on blood agar in Hawassa. These variations could be a result of differences in sample size, use of quarter-level samples, methods employed, and proficiency of laboratory professionals limitations of the culture methods, low level of bacteria in milk, cow pretreated with antibiotics and causative agents of mastitis not bacteria [80].

In case of present study resulted in isolation of numerous pathogenic bacteria. The most dominant pathogenic species that causes clinical and subclinical mastitis in the study area were *S. aureus* (22%) followed by *E. coli* (15.7%). The predominance of *S. aureus* (22%, 95% CI: 16.6-28.5) and *E. coli* (15.7%, 95% CI: 11.2-21.6) was also reported in a study conducted elsewhere in the country [35, 33, 67, 69]. This might be linked with the fact that *Staphylococcus aureus* commonly contaminates the udder and typically results in a minor long-lasting and subclinical disease that is transferred through milk to healthy animals, especially during milking procedures. In addition, *E. coli*, can cause contamination of the udder over bedding, calving stalls and udder washing water [1].

In the present study, the higher prevalence *S. aureus* and *S. intermedius* in clinical mastitis (i e. 29.5% and 6.6%) than in subclinical mastitis (i e. 18.5% and 3.8%) coincides with the findings of [33, 37] who reported a higher proportion of *Staphylococcus aureus* from clinical cases of mastitis as compared to subclinical cases. However, Jafer *et al*. [37] reported a prevalence of *S. aureus* to be 25.7% from Dire Dawa. In contrast, Abebe *et al*. [56] and Christine *et al.* [58] reported a prevalence of 51.2% and 15.7% *S. aureus* from Ethiopia and Kenya, respectively. High rate of *S. aureus* is related to poor milking hygiene, as this pathogen primarily spreads during milking through milkers' hands and towels. Likewise, the higher isolation rate of S. *aureus* may well be due to vast ecological distribution in the mammary gland and skin, its localization intracellularly and in micro abscesses within the udder, and its resistance to antibiotics [1].

The prevalence of *E. coli* (15.7%, 95%CI: 11.2-21.6) is comparable to findings of Abebe *et al*. [35] isolated (17.3%) *E. coli* from mastitic milk in Southern Ethiopia. This finding is lower than finding of Demissie *et al*. [69] isolated (25.7%) of *E. coli* from mastitic milk samples from Wukro Tigrai Region Ethiopia but the result of this finding higher than finding of Dereje *et al.* [81] isolate (6.18%) *E. coli* in Holeta. *K. pneumoniae* (3.1%) and *Enterobacter aerogenes*. (1%) were isolated bacterial pathogens in the current study. The present study results for Klebsiella is aligned with the finding of Zerfu *et al*. [48] isolated (4.6%) in West Hararghe Zone Ethiopia. Moreover, the results for *Enterobacter aerogenes* is aligned with the finding of Christine *et al*. [58] isolated 0.7% in Kenya. Differences between isolation rates of coliform organisms and other environmental mastitis-inducing bacteria may be related to poor farm hygiene, poor slope of stable settings, poor sanitation of milking materials, and absence of use of individual towels. Above all, feces, a typical origin of *E. coli*, can cause contamination of the udder over bedding, calving stalls and udder washing water [1].

In this study Coagulase negative Staphylococcus *(*CNS*)* (10.5%, 95%CI: 6.8-15.7) were the third most predominantly isolated bacteria. This finding align with that of Fesseha *et al.* [33] isolated (12.5%) Coagulase negative Staphylococcus *(*CNS) from mastitic milk from mojo town. However, the isolation rate of Coagulase negative Staphylococcus *(*CNS*)* of this study was higher than finding of Dabele *et al*. [59] isolated (7.9%) of CNS from mastitic milk sample in selected Districts, West Shewa Zone Oromia Ethiopia. but also the current isolation rate (CNS) of this study much lower than the isolation rate of Christine *et al*. [58] isolated a (42.8%) from mastitic milks from keniya. This variation in the frequency of CNS observed from mastitic milk might be attributable to differences in dairy cow breeds, management practices, and laboratory analytical methodologies used in different nations.

The isolation rate of S*treptococcus agalactia* (6.8%, 95%CI: 3.9-11.5) in this study was the fourth predominant bacterial isolate next to *Staphylococcus aureus, E. coli* and coagulase negative staphylococcus. The current finding is comparable to previous finding of Lakew *et al*. [62] isolated (10%) of *Streptococcus agalactia* from mastitic milk and the current finding was much higher than finding of Christine *et al*. [58]. isolated (0.4%) of *Streptococcus agalactia* from mastitic milk but result of this study was lower than result reported by Zeryehun and Abera, [65] isolated (17.1%) of *Streptococcus agalactia* from mastitic milk. The variation of prevalence of *Streptococcus agalactia* could be associated with absence of dry cow therapy, absence of use of individual towels and laboratory analytical methodologies used [82]. In this study the least isolated bacteria were *Micrococci spp, Streptococcus ubris, Enterobacter aerogenes* and *Enterococci spp* which is

in agreement with reports of, [33, 47, 48, 58].

The prevalence of *S. aureus* was significantly associated with the age of cows (P= 0.001). The likelihood of the occurrence of *S. aureus* in adult cows is 5.9 and 1.9 times lower than in old cow and young cow, respectively. This finding was disagree with the finding of Endrias *et al*. [83] who reported not significant association between the occurrence of *S. aureus* with age of cows. This might be older cows have largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agent in the cows' udder [1].

The present finding of an association of *E. coli* mastitis with a history of mastitis was in harmony with other reports [84]. Cows with a history of mastitis were four times more likely to have *E. coli* mastitis than those with no history. The current result may imply that the treatment of cows for mastitis may not be effective in eradicating the pathogens [85]. It could also be due to repeated challenges of the mammary tissues with coliforms coupled with other stress factors resulting in more significant risks of re-infection from the environment [21]. Prevalence of *E. coli* was significantly associated with leg and udder dirtiness (p= 0.005). Likelihood of the occurrence of *E. coli* in cow which had very dirty leg and udder was four times higher than in cow had moderate dirty leg and udder. This finding is in line with previous reports of Abegewi *et al*. [84] who reported high prevalence of *E. coli* in cow which had very dirty leg and udder. This might be moisture, mud and manure present in the environment of the animals are primary sources of exposure for environmental mastitis pathogens [86].

5. Conclusion

The present study revealed that mastitis is the major problem of lactating cows in the dairy farms of Dire Dawa. In addition, this study reported prevalence of mastitis at cow level (24.01%) as well as farm levels (100%). Subclinical mastitis was the major type of mastitis in the area. Mastitis prevalence was influenced animal attributes such as age, parity, udder position, previous mastitis history, and leg and udder dirtiness. Meanwhile, environmental factors such as floor type, and milking mastitic cow last were determinantal for mastitis occurrence. Accordingly, cows aged > 8 years, parity >6 calving, pendulous type of udder, previous history of clinical mastitis, very dirty leg and udder, living in soil floor of barn, and managed in farms with no separate milking for mastitic cow were at high risk of developing mastitis. The study also revealed that various Gram positive and gram bacteria are common among mastitic quarters. From these, *S. aureus, E. coli,* coagulase negative *Staphylococcus*, were frequently isolated from both forms of mastitis, while *Str. agalactiae*, *Str. dysgalactiae, S. intermedius, S. hyicus,* and *K. pneumoniae* were detected to some extent. This showed the involvement of diverse bacterial species in the process of mastitis development. Another notable finding is that the target pathogens were detected in majority (82.7%) of mastitic quarters, from which it was higher in clinically affected quarters (96.7%) than apparently healthy (76.2%), suggesting the importance of these bacteria in the udder inflammation. Cows aged > 8 years, with > 6 calving and with previous history of mastitis were determinant for *S. aureus* occurrence, but age, parity and breed were not determinantal for *E. coli* occurrence in mastitic quarters. It can be concluded that lack of implementation of the routine mastitis prevention and control practices by all of the farms observed and preponderance of the risk factors noted are the main reasons for the observed high prevalence of mastitis in dairy farms. Additionally inadequate sanitation of the dairy setting, poor milking hygiene, and lack of adequate attention to the health of the mammary glands were major factors contributing to the prevalence of mastitis.

Based on the above conclusion, the following points are forwarded as recommendations

1) Warrants the need for applying feasible mastitis intervention strategy with special emphasis on environmental and sub-clinical mastitis.
2) Farm owners and farm attendants should apply regular testing for subclinical mastitis and proper treating of cows affected by sub clinical mastitis pre- and post-milking udder washing, and proper sanitation of bedding should be applied to dairy farms to overcome the problem in the study area.
3) Hygiene of the milkers, milking equipment, and cows in the milking and husbandry system should be considered in attempt to reduce the occurrence of contagious and environmental mastitis in the study area.
4) Slaughtering of chronically affected cows, old cows, cows having many calving and cows which have pendulous udder position.
5) Further research should be needed to view other risk factor of mastitis, identification of other causative agent and antimicrobial drug sensitivity test in study area.

Data Availability

The data sets used and/or analyzed in the current study will be provided from the corresponding author on reasonable request.

Author Contribution

All authors took part in drafting, revising, or critically reviewing the article, gave final approval to the version to be published, have agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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Conflicts of Interest

The authors report no conflicts of interest in this work.

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