Bovine Mastitis: Prevalence, Isolation of Bacterial Species Involved and its Antimicrobial Susceptibility Test around Debrezeit, Ethiopia

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Abstract

A total of 300 local zebu lactating cows of small holders farmers around Debrezeit were examined to determine the prevalence of mastitis with associated risk factor, isolate bacterial pathogens involved and its antimicrobial susceptibility profiles. Clinical prevalence was determined through examination of abnormalities of milk and udder and California Mastitis Test (CMT) were used for determining subclinical mastitis. Bacterial culture and Agar disc diffusion was used for isolation and antibiotic susceptibility test. Based on the result out of 18 (6%) positive samples, 2 (0.7%) were clinical and the other 16 (5.3%) were sub-clinical mastitis. Among the potential risk factors considered, there was significant difference (P<0.05) between semi-intensive and extensive farming system. All positive samples were positive for aerobic bacteria. The bacterial species isolated were CNS and *Micrococcus* species 22.2%, *Staphylococcus aureus* 16% *Staphylococcus epidermis* and *Mycoplasma* species 11.1%, *Enterococcus* species, *Streptococcus agalactiae* and *Staphylococcus hycus* were 5.65%. Comparing the overall efficacy of antimicrobials on isolate kanamycin was the most effective antibiotic where 85.7% of the total isolate were found to be susceptible.

Keywords: Antimicrobial susceptibility; Bacterial pathogen; CMT; Bovine mastitis; Subclinical mastitis

Abbreviations: AWARDO: Ada Woreda Agriculture and Rural Development Office; DARC: Debrezeit Agricultural Research Center; CMT: California Mastitis Test; CNS: Coagulase Negative Staphylococci; FAO: Food and Agricultural Organization; masl: Meter above Sea Level; NVI: National Veterinary Institute; NMC: National Mastitis Council; MOARD: Ministry of Agriculture and Rural Development.

Introduction

Mastitis is the resulted from injurious agents including pathogenic microorganisms, trauma and chemical irritants [1]. According to Quinn et al. [2] over 130 different microorganisms have been isolated from bovine mastitic milk samples, of which almost all are bacteria. The most common pathogen comprises contagious bacteria mainly *S. aureus* and *S. agalactiae* and environmental bacteria mainly coli forms and some species of streptococci that are commonly present in the environment [1,3].

Mastitis infection is spread to teats between milking with organism being transferred up in to udder during milking process itself. *Streptococcus uberis* is the bacteria causing the majority of the problems, due to its resistance to treatment and chronic infections result in high cell count [4,5].

Mastitis causes milk unsuitable for human consumption or provide a mechanism for the spread of diseases like tuberculosis, Streptococcal intoxication, colibacillosis, Streptococcal sore throat and Brucellosis to human [1]. Public hazards associated with the consumption of antibiotic contaminated milk and products cause allergic responses, changes in intestinal flora and development of antibiotic resistant pathogenic bacteria [6].

Besides, mastitis has been recorded as one of the major disease of economic importance in dairy industry worldwide. It causes greater economic loss: much of the losses are related to production lose from inflammation of the infected quarters [4,5]. Owing to the heavy financial implication involved and the inevitable existence of latent infection, mastitis is obviously an important factor that limits dairy production. Evidence to date shows that affected dairy cows may lose 15% of their production and the affected quarter a 30% reduction in productivity. To these gross losses could be added losses associated with its keeping quality and manifesting processes. The disease is also responsible for high percentage of the cows being culled from the dairy herd. Change in milk quality, the possibility of permanent blindness to one or more quarter or even entire udder and death of the cow can resulted from the disease interims of economic loss, it is undoubtedly the most important disease which the dairy industry has to embark upon [1].

In Ethiopia, cows represent the largest proportion of cattle population. According to the food and agriculture organization [7], 42% of the total cattle heads for the private holding are milking cows. Milk produced from these animals provides important dietary sources for the majority of urban and per urban population. However, milk production often doesn't satisfy the country's requirement due to multitude of factors contributing disease of the mammary glands known as mastitis which is among the leading various factors contributing to reduced milk production [8]. According to Lema et al. [9], of the major diseases of cross bred cows in Addis Ababa milk shed, clinical mastitis was the second most frequent disease next to reproductive diseases in which 171 cows out of 556 were found to be affected with mastitis.

The control of mastitis in dairy herds is accomplished in part with the aid of antibiotic; however, it is not the entire answer to the problem. In addition, indiscriminate uses of drugs which are widely used in the country have potential effect on the development of resistant bacteria. Due to complexity of problem of mastitis in Ethiopia the application

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of single preventive and control measure is impractical [10]. Hence scrutinizing the degree of the problem of mastitis in dairy cows, assessments of associated risk factors in relation to the type of mastitis, isolation of bacterial causes and testing for antibiotic susceptibility are the core for looking strategic treatment, control and prevention of the disease so as save economic loss from milk, milk product and culling. Based on the above motive the objectives of the study is to determine the prevalence of Bovine mastitis and associated risk factors, isolate and identify the major mastitis causes bacterial and conduct *in-vitro* antimicrobial susceptibility test of isolates in small holders privately owned Dairy farm around Debre Zeit.

Materials and Methods

Study methodology

Study design and population: Across sectional study was conducted to determine the prevalence of both clinical and subclinical mastitis after a total 300 cow's milk samples were collected by simple random sampling from local indigenous lactating cows that are kept by small holder farmers around Debrezeit town under extensive and semi intensive husbandry system.

Data collection: A structured questioner was developed and all information relating to the study objectives were recorded. Data which was recorded includes type of dairy husbandry system, breed, age, parity and location stage, udder and milk abnormalities (injury, blindness, swelling, milk clots, abnormal secretion etc.) were also recorded during sample collection.

Clinical inspection of udder: The udder was examined through visual inspection and palpation to detect possible fibrosis, consistency of mammary quarters, swelling of super mammary lymph nodes, disproportional symmetry of teats, blindness, discoloration of milk, presence of clots, flakes of blood and watery secretions, increased temperature, pain and disturbance of function [1,2].

Detection of mastitis: Mastitis was detected using the CMT and result of clinical inspection of udder. Equal quantity of milk and CMT reagent was added to the cup on a white paddle and gentle swirling was applied to the mixture in horizontal plane. The result of the test was indicated on the basis of gel formation [11]. The interpretation (grades) of CMT was evocated the result grade as for normal 0 and 1, 2, 3, for positive [2].

Microbial investigation of mastitis

Milk sample collection: Milk was collected before the cow treated with either intra-mammary or systemic antimicrobial agent. For good collection of sample the teats were wiped thoroughly with 70% ethyl alcohol, paying particular attention to the test orifice and removed the first stripe of milk. The sterile collection bottle will be kept in the crook of the little finger so that the lid does not become contaminated. The milk sample was kept refrigeration from the time of collection to the time of bacteriological examination [2].

Direct Microscopy: The milk sample was centrifuged and stained smears from the deposit. A gram-stain is used routinely. Zehil Nelson staining was done when bacteria such as *Mycobacterium bovis* are suspected [2].

Culture: The bacteriological culture was formed following the standard microbiological technique and microbiological procedures for the diagnosis of bovine mastitis infection [2,6]. A loop full of milks streaked on 7% sheep blood agar plates are checked for growth after 24, 48 and up to 72 hours to rule out slow growing microorganisms such as *Corynebacterium* species sample was considered negative if there is no growth after 72 hours. For primary identification, colony size, shape color hemolytic characteristic, gram reactions were considered. These colonies were sub cultured to get pure colonies to nutrient agar, MacConkey agar, Edwards medium etc. other biochemical Estes.

Characterizations of isolated mastitis causing bacteria was done by different methods, biochemical tests such as catalase, oxidase, coagulase, sugar fermentation test, oxidation fermentation test and indole test. The procedures adapted from Quinn et al. [2] for the identified pathogens were used.

Antimicrobial sensitively testing: Selected bacterial isolates were tested for sensitivity to different antimicrobials. Using *in vitro* disk diffusion (Kirby-Baur) method as described in [2]. Cultured broth was cross-checked with McFarland to Mueller Hinton agar and disc application. After measuring the zone of inhabitation, it was classified as sensitive, intermediate and resistant according to national committee for clinical laboratory standard (NCCLS) [2].

Statistical analysis

SPSS version 20 was used to analyze the collected data. The association among and between the considered risk factors were tested by using Chi square (χ^2) and Odds ratio (OR). The level of significance was also expressed using P-value less than 0.05.

Results and Discussion

Prevalence of clinical and sub clinical mastitis

A total 300 indigenous zebu lactating cows from small holder dairy farm around Debrezeit were examined to determine the prevalence of bovine mastitis. Out of 300 examined cows there was high prevalence of sub clinical mastitis 16(5.3%) than clinical 2(0.7%) as shown in Table 1. A total of 1200 quarters were examined and 72(6%) were CMT positive whereas 33(11.1%) were found blind teats (Table 2).

Prevalence of mastitis using multivariate risk factors

The prevalence of mastitis using multivariate risk factors was shown in Table 3. Those cows with different lactation stage and farming system revealed significant difference (p<0.05) in prevalence of mastitis (p>0.05). The prevalence of both clinical and sub-clinical mastitis by risk factors were insignificance (P>0.05) with treatment history, lactation stage and parity however, there was significance difference between semi intensive and extensive farming system (P<0.05). The proportions of sub-clinical mastitis were higher than clinical one within all current considered risk factors Table 4.

A total of 18 cows that were positive both clinically and sub clinically, all samples were collected and cultured. From 18 cultured samples, 18(100%) samples were positive for aerobic bacteria. The isolated bacterial species and were presented below in Table 5. Coagulase Negative *Staphylococcus* (CNS) and *Micrococcus* species were

Number of cows examined	Clinical mastitis	Sub-clinical mastitis	Total
	Positive No. (%)	Positive No. (%)	
300	2(0.7%)	16(5.3%)	18(0.4%)

Table 1: Prevalence of clinical and sub clinical bovine mastitis at Cow level using CMT.

Quarter	CMT Positive No (%)	CMT Negative No (%)	Blind No (%)
Left Back	28(2.33)	1172(97.7)	11(1.2)
Left Front	24(2)	1176(98)	3(0)
Right back	12(1)	1188(98.7)	17(1.2)
Right Front	8(0.67)	1192(99.3)	2(0.8)
Over all	72(6)	1128(94)	33(11.1)

Table 2: Quarter level of prevalence of mastitis and blind teats.

Risk factor	No of cow Examined	Positive No (%)	X^2	p-value
Location stage				
Early	108	7 (6.4)		
Mid	128	3(16.7)	7.873	0.020
Late	64	8 (44.4)		
Age (yr)				
3.5-6	134	8(44.5)		
6-9	133	6(33.3)	1.85	0.229
9-13	33	4(22.2)		
Parity 1-3	138	6(33.3)		
3-6	84	5(27.8)	1.892	0.388
6-9	78	7(38.9)		
Farm system				
Extensive	293	14(77.7)	39.953	0.000
Semi intensive	7	4(22.2)		
History treatment				
Treated	187	10(55.6)	0.338	0.561
Untreated	113	8(44.4)		

Table 3: The prevalence of mastitis within the considered risk factors.

Risk factors		Type of mastitis positive		X^2	p-value
		Clinical No (%)	Sub clinical No (%)		
Treatment history					
Treated	114	1(50)	9(56.2)	0.367	
Untreated	186	1(50)	7(43.8)		0.832
Location stage					
Early	108	1(50)	6(37.5)		
Mid	128	0(0)	3(18.8)	8.129	0.089
Late	64	1(50)	7(43.8)		
Age (year)					
3.5-6	134	1(50)	(57.8)		
6-9	133	1(50)	62(54.9)	4.145	0.387
9-13	33	0(0)	35(53.0)		
Parity 1-3	138	1(50)	6(37.5)		
3-6	84	1(50)	4(25)	1.892	0.388
6-9	78	0(0)	6(37.5)		
Farm system Extensive	293	2(100)	12(75)	45.622	0.000
Semi intensive	7	0(0)	4(25)		

Table 4: Prevalence of clinical and sub clinical mastitis in different risk factor.

isolated with high prevalence (22.2%), *Staphylococcus aureus* 16.7%, *Staphylococcus intermedius* and *Mycoplasma* species were 11.1%, and *Streptococcus agalactiae*, *Enterococcus* species and *Staphylococcus hycus* were 5.6%.

From the tested bacterial isolate using selected antimicrobial agent all are susceptible for kanamycin and except one isolate of CNS. CNS shows intermediate (50%) for both Chloramphinacol and Kanamycin as shown in Table 6.

Discussion

In this study, the overall bovine mastitis prevalence is 6%. The prevalence is very less compared to the others study finding done on mastitis elsewhere in Ethiopia such as 52.27% by Abunna et al. [12], 34.9% by Biffa et al. [13], 74.7% by Zeryehun et al. [14] and Ashenafi [15] who reported 75% prevalence. This might be due to management system the farmers have practiced in the study area. Farmers have maximum of cow 2-3 and they release their cows to open grazing land this system decreases the chance of transmission of contagious mastitis between cows [1]. Up on taking history on the cows' production of milk, they only give less than 1.5 liter/day. Local Zebu breed are low in milk production and resistant to mastitis [16]. Higher yielding cows have been found more susceptible to mastitis owing to position of teats, udder, and anatomy of teat cannel making them prone to injury, and due to fewer efficacies of pathogenic cells in higher yielding cows associated to dilution [1,17].

The clinical mastitis prevalence in this study was 0.7% which was comparable with that of Gizat [18], who reported 0% in local Zebu lactating cows in and around Bahir Dar. However, the present finding was lower than that of Tesfaye [19], who reported 7.3% in Adama, and Adugna [20], who reported 5.7% in Dire Dawa and Haramaya University Dairy farm. Most of the time when comparing clinical and sub-clinical mastitis, clinical mastitis is lower than that of sub-clinical mastitis and this is because of treatment of clinical mastitis is commonly practiced [19].

In Ethiopia, the sub-clinical mastitis received little attention and efforts have been concentrated on the treatment of clinical cases while the high economic loss could come from sub-clinical mastitis [21]. The present finding of prevalence of sub-clinical mastitis was 5.3% which is comparable with that of Tesfaye [19] who reported 7.3% in Adama and less than that of Adugna [20] who reported 18.9% in Dire Dawa and Haramaya University Dairy farm and Gizat [18] who reported 17% in and around Bahir Dar on the local Zebu lactating cows. In the present study, as well in other conducted studies, over whelming cases of mastitis were sub-clinical compared to clinical mastitis [22,23].

Dairy farms in the study area usually complain about the decrease in milk yield irrespective of adequate feed provision and deworming practice. The high prevalence of sub-clinical mastitis may be due to attributed to improper milking hygiene, poor house hygiene, lack of post milking teat dipping and practicing of milk by contact labors use of lubricant, absence of order in milking cows of different ages. Moreover, its occurrence was high in dairy farms without noticeable in farm treatment as Radostits et al. [1] and Quinn et al. [2] provide the same reasoning.

The prevalence of mastitis which was not significantly influenced by considered potential risk factor (P>0.05) are age, parities, and treatment history. But lactation stage and farming system are significantly the potential risk factors. This could be due to as lactation stage increases chance of cows exposed to the mastitis increases because daily contact of cows while milking and in farming system with no care milking management.

From total examined 1200 quarters 72(6%) quarter were CMT positive and 33 (11.1%) blind teats. Blindness of the teat could be due to failure to detect the disease in early stage attributable to lack of strip cup examination and skill milker's to establish a prompt treatment. Generally, it indicates a poor treatment regime and husbandry [6].

Analysis of the bacteriological examination of the milk samples was made to isolate and identify the main etiological agents involved in the disease process. The organisms were identified on the bases of their culture, staining characteristics and biochemical reactions. The result of bacteriological analysis in the present study showed that Coagulase Negative *Staphylococcus* and *Micrococcus* species were predominant pathogens which were 22.2% of all the isolated bacterial species. The high isolation rate of CNS in the study could be associated with it characteristics of chronicity. The CNS isolated was all found from sub-clinical mastitis. However, CNS was isolated from bovine and other dairy animals' mastitic milk samples which indicated that they could be pathogenic even causes more mastitis than *S. aureus* [24]. According to Pyorolla, over 30% sub-clinical and nearly 20% of acute cases of mastitis were usually due to CNS which is in agreement with the present result (22.2%). The isolated *Micrococcus* species (22.2%) was comparable with the finding of Amen et al. who reported 26.67% in different parts of Ethiopia and it was higher than the findings of Tarekegn [6], Gizat [18] and Mekonnen et al. [25] who reported 8.15, 5.2 and 10.2% respectively.

The isolation rate of *S. aureus* 16.7% in the present study is the second next to CNS and Micrococcus species and closely comparable with the findings of Gizat [18]. Bishi [24] and Hussein [21] reported 17.8% and 9% in Addis Ababa respectively. However, the present finding is lower than that of Workineh et al. [22] and Kerro and Tereke

No.	Isolates Frequency	No (%)
1.	*Staphylococcus aureus* 3	16.7%
2.	Micrococcus Species 4	22.2%
2.	*Staphylococcus hycus* 1	5.6%
4	*Staphylococcus intermedius* 2	11.1%
5.	*Streptococcus agalactiae* 1	5.6%
6.	*Mycoplasma species* 2	11.1%
7.	Coagulase Negative *Staphylococcus* 4	22.2%
8.	*Enterococcus* species 1	5.6%
Total	**18**	**100%**

Table 5: Isolated bacteria species from CMT Positive milk sample.

Response to application of antimicrobial disks (%)																
Bacteria isolates	N	C_{30}			AP_{10}			TE_{30}			AX_{10}			K_{30}		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
S. aureus	2	1(50%)	1(50%)			2(100%)	-	-	-	2(100%)	1(50%)	1(50%)	-	2(100%)	-	-
S. intermedius	1	1(100%)	-	-	1(100%)	-	-	-	1(100%)	-	1(100%)	-	-	1(100%)	-	-
Micrococcus Sp.	2	1(50%)	1(50%)	-	1(50%)	1(50%	-	2(100%)	-	-	2(100%)	-	-	2(100%)	-	-
CNS	2	-	1(50%)	1(50%)	-	-	-	2(100%)	-	-	2(100%)	-	-	-	2(100%)	-
S. hycus	1	-	1(100%)	-	1(100%)	1(100%	-		-	-	-	-	1(100%)	1(100%)	-	-
Enterococci	1	1(100%)	-	-	-	-	-	-	-	-	-	1(100%)	-	1(100%)	-	-
S. agalactae	1	-	1(100%)	-	1(100%)	-	-	1(100%)	-	1(100%)	-	-	-	1(100%)	-	-

Keys: N: Number of observations; AX: Amoxicillin; C: Chloramphnicol; I: Intermediate K: Kanamycin, R: Resistance, S: Susceptible, TE: Tetracycline

Table 6: Antibiotic sensitivity testing.

[10] where *S. aureus* account 39.2 and 40.5% isolates respectively in Addis Ababa and Sothern Ethiopia. The relatively high prevalence of *S. aureus* in this study could be associated with total absence of dry cow therapy and post milking teat dipping, the invariable hold milking practice and low culling rate of chronically infected cows. Culling was usually due to feed shortage, ageing and reproductive problem. *Staphylococcus intermidus* and *Staphylococcus hycus* were found in the rate of 11.1 and 5.6% respectively.

Streptococcus agalactiae was found 5.6% and this bacterial species was lower isolate in the study area and this could be due to management system in the study area. Environmental Streptococci may be due to poor housing facilities which predispose to the accumulation of feces on cows which could increase the rate of exposure of the teats and udder to the pathogens. *Mycoplasma* species was found with rate of 11.1% in the study farms. Mycoplasmas are group of very small organisms that can be cultured from multiple body sites of both sick and healthy cattle. Some common Mycoplasma include *M. bovis* most commonly cultured from the udder [26]. The organisms were isolated from clinical infected cows which agree with the finding of Thomas [26]. In milk samples obtained from individual cows, a negative Mycoplasma culture usually means that the organism is not present. However, intermittent shedding of organism has been reported, so false negative cultures may rarely occur [27].

When comparing the overall efficacy of antimicrobials on isolates, kanamycin was the most effective antibiotic where 85.7% of the total isolate were found to be susceptible. The drug was not used in the study area for the treatment of animals. *S. aureus* was 100% resistant to Tetracycline. The development of antibiotic resistance nearly always has followed the continuous and under dosage use of the antimicrobial agents [2].

The dominant pathogens in the study area were CNS and Micrococcus species. CNS isolate were 100% susceptible to Tetracycline, Amoxicillin and Kanamycin however intermediate to Chloramphenicol and Ampicillin (50%) which is in agreement with the report by Bishi [24].

In this study *S. aureus* was the most susceptible to Kanamycin and Ampicillin and intermediate to Chloramphenicol, and Amoxicillin 100 and 50% respectively. A report of Bishi [24] indicated that Tetracycline was not effective in *S. aureus*.

Micrococcus species were 100% susceptible to Tetracycline, Amoxicillin and Kanamycin. This might be due to non-drug treatment with Amoxicillin and Kanamycin in the study areas [28]. *Staphylococcus hycus*, *Staphylococcus intermidius* and Enterococci species were also 100% susceptible to Ampicillin, Tetracycline and Kanamycin.

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Conflict of Interest

I declare that this paper presents the work carried out by myself and does not incorporate with the acknowledgement of any material and finance.

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