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Bovine Mastitis: Prevalence, Risk Factors, Major Pathogens and Antimicrobial Susceptibility Test on the Isolates around Addis Ababa, Central Ethiopia

Tesfaye Belachew*

Assela Regional Laboratory, Animal Health Diagnostic, Oromia, Ethiopia

***Corresponding Author:** Tesfaye Belachew, Assela Regional Laboratory, Animal Health Diagnostic, Oromia, Ethiopia.

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Abstract

Livestock represent a major national resource and form an integral part of the agricultural production system. Ethiopia holds large potential for dairy development due to its large livestock population and the favorable climate for improved and high yielding breeds

This cross- sectional study was conducted on lactating cows in Sululta and Bareh districts of Finfinnee surrounding special zone of Oromia regional state, Central Ethiopia. Sululta district is located 40 km from Addis Ababa with an altitude ranges from 2100 to 2593 meters above sea level (m.a.s.l).

The milk samples were taken from cows not treated early with either intra mammary or systematic antimicrobials agents. For good collection of sample, the teat was wiped thoroughly with 75% ethyl alcohol

From currently tested few isolates using selected antimicrobial agents, all susceptible for Chloramphenicol and Gentamycin except one each isolates of *Staphylococcus aureus* and *micrococcus species* for both and *Corynebacteriumpyogenes* for Chloramphenicol and *Coagulase Negative Staphylococcus* for Gentamycin which shows intermediate. Similarly, most of the isolates susceptible to Amoxycillin except some isolates of *Staphylococcus aureus, Streptococcus uteri's, Coagulase negative Staphylococcus* and *Corynebacteriumpyogenes* Prevalence of mastitis, particularly, the subclinical one could bring about major economic losses in dairy cows without notice as result of reduced milk production, poor growth or mortality of suckling calves and dam health.

Keywords: *Clinical Mastitis; Subclinical Clinical Mastitis; Antimicrobial; Susceptible; Isolates*

Abbreviations: CMT: California Mastitis Test; CNS: Coagulase Negative Staphylococci; Masl: Meter above Sea Level; NMC: National Mastitis Count

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Introduction

Livestock represents a major national resource and constitutes an integral part of the agricultural production system. Ethiopia holds great potential for dairy development due to its large livestock population and favorable climatic conditions supporting improved and high yielding breeds. Given the considerable potential for generation of income and employment, the development of smallholder dairy sector in Ethiopia, has a promising future and can contribute significantly to the alleviation of poverty and improve the nutritional status of the country. According to [1] milk production during the 1990s expanded at an annual rate of 3.0% compared to 1.63-1.66% during the preceding three decades, with the expected growth in income, increased urbanization and improved policy environment [2]. In Ethiopia, it is estimated that 82% of the milk is supplied unpasteurized by intra and peri-urban producers to consumers, while only 18% is supplied by dairy enterprises in pasteurized form [3].

Mastitis is an inflammation of the mammary gland, primarily resulting from the invasion of the mammary gland by pathogenic microorganisms through the teat canal resulting in physical, chemical, pathological and bacteriological changes in glandular tissues and milk. Mastitis has been recorded as one of the major diseases of economic importance in the dairy industry worldwide. It causes greater economic loss; such that a larger proportion of the losses are attributed to production loss due to the inflammation of the infected quarters [4].

Owing to the heavy financial implications involved and the inevitable existence of latent infection, mastitis is obviously an important factor that limits dairy production. Evidence to date shows that the affected dairy cows may cause a loss of 15% in production and the affected quarter, a 30% reduction in productivity. To these gross losses could be added losses associated with the related keeping quality and manufacturing processes [5].

The most common pathogen associated with mastitis includes the contagious bacteria mainly *S.aureus* and *S.agalactae* and environmental bacteria mainly coli forms and some species of streptococci that are commonly present in the environment [6].

Bale is a high potential cereal-livestock zone where dairy activity plays a significant role in the livelihood of farmers residing in the area. Due to the above-mentioned reasons and the economic capacity of the peasants' smallholder dairy farms, production involving crossbred dairy cattle is a common practice in the area. Yet, adequate information on the prevalence of subclinical mastitis in the area is absent and what is available is fragments of information from cases of clinical mastitis that has been presented to the veterinary clinic for treatment.

Therefore, objectives of the study were:

- To determine the prevalence of Bovine mastitis and association of risk factors in small holders privately owned Dairy farm around Addis Ababa
- To isolate and identify the major bacterial mastitis To conduct *in-vitro* antimicrobial susceptibility test of the bacterial isolates to commonly used antimicrobials

Materials and Methods

Study area

This cross-sectional study was conducted on lactating cows in the Sululta and Bareh districts of Finfinne surrounding the special zone of Oromia regional state, Central Ethiopia. Solute district is located 40 km away from Addis Ababa with an altitude that ranges from 2100 to 2593 meters above the sea level (m.a.s.l). And Bareh Sululta district is located 30 km from Addis Ababa with an altitude that ranges from 2000 to 2514 meters above the sea level (m.a.s.l).

Study population

Study was conducted in Sululta and Bareh districts of Finfinne, surrounding special zones of Oromia regional state. A total of 351 heads of cross and local breeds of lactating cows were kept under small dairy holder herds under extensive and semi-extensive husbandry practice.

Study design and period

The study of bovine mastititis was performed using cross-sectional study on cows and quarters to analyse the clinical manifestations for clinical mastitis and indirect test (California mastitis test and culture) for sub-clinical mastitis.

Ample size determination and sampling strategy

The sample size was calculated according to the formula given by [7]. It is calculated by considering (89.54%) the estimated prevalence from previous report by [8] Therefore, the calculated sample size included 144 lactating cows from each district, but to increase the probability of getting positive lactating cows, 63 cows were taken additionally. Thus, the total sample size was 351 with a 95% confidence level and 5% precision level. Simple random sampling method was employed to select the dairy cows individually.

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

Where n = require sample size, Pexp = expected prevalence, d = desired absolute precision

Study methodology

Data related to different potential risk factors (age, parity, and lactation stage, housing conditions, previous history of mastitis and husbandry system) were collected for 351 lactating cows from farm records which were available and by interviewing the cows' owner when not available. Clinical examination of the udder, screening using the California mastitis test (CMT) and bacteriological examination also were carried out.

3.5.1. Data collection

Data on each sampled cow were collected in properly designed form (Annex 1).

Clinical inspection of the udder

The clinical examination of the udder was performed visually, followed by palpation to detect possible swelling, pain, and disproportional symmetry, blindness of teats and discoloration of milk due to mastitis [9].

Detection of mastitis

The Californian mastitis reagent was used to screen cows with subclinical mastitis such that milk sample collection was performed according to the procedures recommended by the National Mastitis Council (10). The results of the test were indicated based on gel formation. The interpretation (grades) of the CMT was evocated and the results graded as 0 for negative and trace 1, 2 and 3, for positive [9] Annex 2.

Microbial investigation of mastitis

3.6.1. Milk sample collection

The milk samples were taken from cows not treated earlier with either intramammary or systematic antimicrobial agents. For ensuring good collection of milk sample, the teat was wiped thoroughly with 75% ethyl alcohol. A sterile collection bottle was used and the

first streams of milk from each quarter were discarded. The milk sample was then held in an ice box for transportation to the laboratory. In the laboratory, samples were cultured immediately or stored at +4°C [10].

Direct microscopy

The milk sample was centrifuged and a smear made from the deposit was stained. A Gram stain was used routinely. The Ziehl Neelson staining is performed for rare cases when bacteria such as *M. bovis* are suspected to be present in the sample [9].

Culture

Before milking, milk samples were collected aseptically for microbiological cultures, according to the procedures of the [10]. Culturing of milk samples collected from individual cows, to identify mastitis producing organisms as standards of examination for mastitis [6]. The bacteriological culture will be performed following the microbiological technique [9] and microbiological producers determined for the diagnosis of bovine mastitis infection [10].

Antimicrobial sensitivity testing

Selected isolates were tested for their sensitivity to different antimicrobials which are used commonly in the area. In vitro diffusion (Kirby-Baur method) as described in (Quinn., *et al*. 2002), was the procedure implemented in the preparation of the inoculation of the 6-hour cultured broth cross-checked with McFarland, using the Mueller Hinton agar and disc application. After analyzing the zone of inhibition, it was classified as sensitive, intermediate and resistant, according to the National Committee for Clinical Laboratory Standard (NCCLS) break point to interpret the inhibition zone [9].

Data Management and Analysis

Data which was collected through, history, clinical inspection, CMT, pathogenic bacteria isolation and identification and antimicrobial sensitivity test result was entered into the data base management software Microsoft Excel computer program and analyzed using SPSS 20 version. The prevalence was expressed using percentage. The association among and between the considered risk factors were tested using Chi square (χ^2), Odds ratio (OR) and determination of OR Confidence interval. The significance of association was also expressed using p-value at 0.05.

Result

Prevalence

A total of 351 lactation cows were investigated during the study period. Out of the total 351 lactation cows, the overall prevalence of mastitis was observed in 80 affected cows (22.79%) (Table 1), where the lower 5 (1.99%) and higher 73 (20.79%) were respectively diagnosed by clinical and subclinical type of mastitis. The prevalence of mastitis was lower 20.94% and higher 24.13% in Sululta and Bareh districts respectively. From the examined lactating cows, 7.40% of the affected cows had at least 1 blind teat. As shown in Table 2, out of 1404 quarters examined during the study period 130 (9.25%) were tested positive for mastitis while 49 (3.49%) had blind teats.

Districts	Mastitis condition		Total No. Examined n (%)	Results n (%)	At least 1 teat blind cow
	Clinical n (%)	Subclinical n (%)			
Sululta	4 (2.70)	27 (7.69)	148 (42.16)	31 (20.94)	17 (11.48)
Bareh	3 (2.17)	46 (38.59)	203 (57.83)	49 (24.13)	9 (4.43)
Total	5 (1.99)	73 (20.79)	351 (100)	80 (22.79)	26 (7.40)

Table 1: *Prevalence of Clinical and subclinical mastitis during study period.*

Quarter	No Examined		Blind No (%)
	Negative (%)	Positive No (%)	
Right back	351300 (85.84)	35 (9.97)	16 (4.55)
Right front	351313 (89.17)	29 (8.26)	9 (2.54)
Left back	351299 (85.18)	35 (9.97)	17 (4.84)
Left front	351313 (89.17)	31 (8.83)	7(1.99)
Over all	1404 1225 (87.25)	130 (9.25)	49 (3.49)

Table 2: *Quarter level prevalence of mastitis during study period.*

Bacterial isolation and Antibiotic Sensitivity testing

From cultured samples, the bacteria genera/species and isolated were as in (table 3) with high prevalence were *Staphylococcus aureus* (46.63%), *Coagulase negative Staphylococcus* (13.47%) and *Corynebacterium bovis*, *Streptococcus uberis* and *Micrococcus spp*. (5.88%).

No.	Types of bacterial isolates	Number (%)
1.	*Staphylococcus aureus*	90 (46.63)
2.	*Streptococcus uberis*	11 (5.88)
3.	*Streptococcus intermidius*	7 (3.74)
4	*Str. agalactae*	4 (2.07)
5.	*CNS (Coagulase negative Staphylococcus)*	26 (13.47)
6.	*Micrococcus spp*	11 (5.88)
7.	*Bacillus cerues*	7 (3.74)
8.	*Proteus Spp*	8 (5.00)
9.	*Corynebacteriumbovis*	11 (5.88)
10.	*Corynebacteriumpyogenes*	9 (4.81)
11.	*Psudotuberculosis*	5 (3.12)
12.	*E. coli*	4 (2.13)
Total		193 (100)

Table 3: *Bacterial isolates during all season.*

As shown in (Table 4) below, from the currently tested isolates using selected antimicrobial agents, all micro-organisms susceptible to Chloramphenicol and Gentamycin except one isolate each of *S.aureus and micrococcus spp* which was sensitive to both. *C. pyogenes* was sensitive to Chloramphenicol and Coagulase Negative *Staphylococcus* to Gentamycin showing intermediate sensitivity. Similarly, most of the isolates were susceptible to Amoxycillin except some isolates of *Staphylococcus aureus, Streptococcus uberis, Coagulase negative Staphylococcus* and *Corynebacteriumpyogenes*. However, high resistance was observed among most of the isolates towards Penicillin.

Response to application of antimicrobial disks (%)																	
Bacterial isolates	**N**	**C30**			**CN10**			**TE30**			**P10**			**AML25**			
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	
S. aureus	64	59 (92.18)	5 (7.81)	-	58 (90.62)	6 (9.37)	-	20 (31.25)	14 (21.87)	30 (46.87)	19 (29.68)	13 (20.31)	32 (50)	39 (60.93)	15 (23.43)	10 (15.62)	
Streptococci uberis	5	5 (100)	-	-	5 (100)	-	-	2 (40)	-	3 (60)	1 (20)	-	4 (80)	2 (40)	1 (20)	2 (40)	
S. intermidius	2	2 (100)	-	-	2 (100)	-	-	-	1(50)	1 (50)	-	-	2 (100)	2 (100)	-	-	
CNS	15	15 (100)	-	-	14 (93.33)	1 (6.66)	-	5 (33.33)	2 (13.33)	8 (53.33)	2 (13.33)	4 (26.66)	9 (60)	7 (46.66)	2 (13.33)	6 (40)	
micrococcus spp	5	3 (60)	2 (40)	-	4 (80)	1 (20)	-	2 (40)	1 (20)	2 (40)	2 (40)	-	3 (60)	4 (80)	1 (20)	-	
B. cereus	2	2 (100)	-	-	2 (100)	-	-	-	2 (100)	-	1 (100)	-	1 (50)	1 (50)	1 (50)	-	
Proteus Sps	2	2 (100)	-	-	2 (100)	-	-	-	-	2 (100)	-	-	2 (100)	2 (100)	-	-	
C. bovis	4	4 (100)	-	-	4 (100)	-	-	-	2 (50)	2 (50)	2 (50)	-	2 (50)	3 (75)	1 (25)		
C. pyogenes	3	2 (66.66)	1 (33.33)	-	3 (100)	-	-	1 (33.33)	2 (66.66)	-	1 (33.33)	-	2 (66.66)	2 (66.66)	-	1 (33.33)	
Str. agalactae	1	1 (100)	-	-	1 (100)	-	-	-	1 (100)	-	-	-	1 (100)	1 (100)	-	-	
E. coli	2	2 (100)	-	-	2 (100)	-	-	-	1 (50)	1 (50)	-	1 (50)	1 (50)	1 (50)	1 (50)	-	
Psudotuberculosis	1	1 (100)	-	-	1 (100)	-	-	1 (100)	-	-	-	-	1 (100)	1 (100)	-	-	
Total	106																

Keys: N = Number of observations, S: Susceptible, I = Intermediate, R = Resistance, C = Chloramphenicol, CN = Gentamycin, TE = Tetracycline = Penicillin, AML = Amoxycilli

Table 4: *Antibiotic sensitivity testing.*

Discussion

In this study, the overall bovine mastitis prevalence was observed among 80 (22.79%) affected cows. The prevalence was higher compared to other studies such that findings on mastitis elsewhere in Ethiopia was observed as 52.27% by [11], 34.9%, 74.7% by Zerehun [12] and [13] who reported 75% prevalence. This might be due to the management system of the farmers who practiced in the study area. On reviewing the history of the cows' milk production, it was understood that they gave less than 2-liter milk/day. The Local Zebu breed is low in milk production and resistant to mastitis [14]. Higher yielding cows have been found to be more susceptible to mastitis

owing to the position of teats, udder, and the anatomy of the teat canal making them prone to injury, due to fewer efficacies of pathogenic cells with respect to dilution in higher yielding cows [6,15].

The clinical mastitis prevalence in this study was 5 (1.99%) which was comparable with that of [16], who reported 7.3% in Adama, and [17], who reported 5.7% in Dire Dawa and Haramaya University Dairy farm. Most of the time when comparing clinical and subclinical mastitis, the incidence of clinical mastitis is lower than that of subclinical mastitis because the treatment of clinical mastitis is commonly practiced [16].

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 subclinical mastitis may be attributed to improper milking hygiene, poor house hygiene, lack of post milking teat dipping and the practice of use of lubricant by contact labors, absence of order in milking cows of different ages. Moreover, its occurrence was high in dairy farms without being attended to in the farm treatment as [6] and [9] provide the same reasons.

Conclusion

From currently tested few isolates using selected antimicrobial agents, all micro-organisms susceptible to *Chloramphenicol* and Gentamycin except one isolate each of *S. aureus* and *micrococcus spp* sensitive towards both the agents, *C. pyogenes* for Chloramphenicol and CNS for Gentamycin which shows intermediate sensitivity. Similarly, most of the isolates were susceptible to Amoxycillin except some isolates of *S. aureus, Str. uberis, CNS* and *C. pyogenes*. However, high resistance was observed by most of the isolates towards Penicillin and tetracycline, which are drugs currently in use for mastitis therapy in the study area.

Prevalence of mastitis, particularly, the subclinical one could bring about major economic losses in dairy cows without notice, because of reduced milk production, poor growth or mortality of suckling calves and ill-health.

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Conflict of Interest

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

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