

## Bovine Mastitis: Prevalence and Isolation of Major Pathogens in Dairy Farms of Selected Sites in Addis Ababa, Ethiopia

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**Abstract:** A cross sectional study was carried out from November 2011 to April 2012 to determine the prevalence of mastitis and to assess associated bacterial pathogens in lactating dairy cows in five selected sub-cities of Addis Ababa (Akaki kality, Nifas silk lafto, Kolfe keraniho, Bole and Yeka). A total of 255 Holstein Friesian and 85 Jersey from exotic breed and 25 local zebu breeds milking cows were tested using California Mastitis Test (CMT). Prevalence of mastitis at cow level was 52.6% (192/365), out of which 14.6% (28/192) and 85.4% (164/192) were clinical and subclinical, respectively. An overall found quarter level prevalence was 29.8% (421/1412). From 127 culture positive samples, a total of 124 bacterial isolates were recovered, the most prevalent being *Staphylococcus aureus* (24.4%) followed by Coagulase negative *Staphylococcus* (CNS) (18.1%). Other bacterial isolates included *S. agalactia* (15%), other *Streptococcus* spp. (11.9%), *Escherichia coli* (11.8%) and *Pasteurella* spp. (3.1%), *Klebsiella* spp. (5.5%), *Corynebacterium* spp. (3.1%) and *Salmonella* spp. (4.7%) also isolated. In general, this study revealed the importance of mastitis and associated bacterial pathogen in the study area which indicates appropriate control measures should be taken to reduce its impact on dairy production in the study area.

**Key words:** Bovine mastitis • Prevalence • Risk factors • *Staphylococcus aureus* • *Streptococcus* spp. • Bacteria

### INTRODUCTION

World have about 1,287,520,000 head cattle out of these dairy consists of 225,502,000 heads. In Africa there are 192,180,000 total populations of cattle, among these dairy consists of 34,057,000 in number. In Ethiopia 49.3 million heads of genetically distinct cattle of which 9.9 million are dairy cows [1]. Despite Ethiopia is the most populous country in cattle than any African country, up to 1997 the per capita milk consumption was 16 kg which was lower than other countries in the region [2]. In recent time Ethiopia is chiefly an agricultural country whose livelihood is heavily depend on crop and livestock production. Among these livestock products milk is one of the most important food items containing more than 200 ingredients. Dairy production is a biologically efficient system that converts feed and roughages to milk which supplies body building proteins, bone forming minerals, health giving vitamins and furnishes energy giving lactose and milk fat [3].

The increase in human population, accessibility to technology input, high demand for animal products and purchasing power in urban center had helped the urban and peri urban dairy farm in the country to flourish [4]. FAO estimated that 42% of the total cattle herds, for the private holdings are milking cows [5]. However, milk production often does not satisfy the country's milk requirements due to a multitude of associated factors including mastitis.

Mastitis is an inflammation of parenchyma of mammary gland regardless of the cause. It is resulted from injurious agent including pathogenic organism, trauma and chemical irritants [6]. A cow or a quarter was considered clinically sick for mastitis when abnormality was observed in milk (like the presence of flakes, clots, bloody or watery appearance), in the udder (like swelling, pain, hotness) or in the cow (systemic signs together with the above manifestations). A cow or a quarter was considered to have subclinical mastitis intra mammary infections if California Mastitis Test CMT score was 1, 2, 3 or when a mastitis pathogen was isolated [7].

Mastitis, known to be a complex and costly disease of dairy cows, that results from the interaction of the cow and environment including milking machine and microorganisms [8]. 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 to cause culling of animals at the age of high producing capacity [9]. Moreover, due to its latent form, heavy financial losses and great nutritional and technological impacts can be resulted. Because valuable components of the milk like lactose, fat and casein are decreased while undesirable components like ions and enzymes are increased and making the milk unfit for processing technology [10].

Milking potential of the cow for the remainder of lactation was estimated at 60 pound for each case [11]. In United States, economic losses from mastitis have been calculated at approximately 200 dollar per/cow per year or 2 billion per year for the nation [12]. Mungube, [13] estimated the economic losses from mastitis in the urban and peri urban areas of Addis Ababa, Ethiopia, to be 210.8 birr per cow per lactation. In addition to its economic impact, there is a danger that the bacterial contamination of milk from affected cows may render it unsuitable for human consumption by causing food poisoning or in rare cases provide a mechanism of spread of disease to humans. Tuberculosis and streptococcal sore throat may be spread in this way [14].

Many infectious agents have been implicated as cause of mastitis in cattle. The most common organisms are *Streptococcus agalactia* and *Staphylococcus aureus* [15], whereas, environmental mastitis is associated with coliforms and environmental Streptococci that are frequently found in the cows environment [16]. Mastitis as a disease, especially the subclinical form, has received little attention in Ethiopia; efforts have only been concentrated to treat clinical cases [17]. Some studies have been conducted so far on the prevalence and the major causes of bovine mastitis in the country [18,19].

In Ethiopia, the available information indicated that bovine mastitis is one of the most frequently encountered diseases of dairy cows. According to Lemma *et al.* [20] of the major diseases of crossbreed cows in Addis Ababa milk shed, clinical mastitis was the second most frequent disease next to reproductive diseases. Generally, the prevalence of clinical and subclinical mastitis in different parts of Ethiopia range from 1.2 to 21.5% and 19 to 46.6%, respectively [13, 20, 21, 22]. These limited studies showed that bovine mastitis is among the problems hindering dairy productivity in Ethiopia and this requires the development of methodologies of control program under the prevailing husbandry system. The disease generally

involves interplay between management practice and infectious agents. Bovine mastitis is the most important disease of dairy cows that hinder dairy production with significant financial implications. Therefore this study was conducted with the objectives to determine the prevalence of mastitis in lactating dairy cow and isolate major bacterial pathogens from milk samples of mastitic cows in the study area.

## MATERIALS AND METHODS

**Study Area Description:** The study was conducted in five purposively selected sub cities of Addis Ababa (Nefas silk lafto, Kolfe keraniho, Yeka, Bole and Akaki kaliti) capital city of Federal Democratic Republic of Ethiopia. According to 2007 population census the city has a total population of 2,739,551 of which 1,305,387 men and 1,434,164 were women on the area coverage of 530.12 km<sup>2</sup>. Addis Ababa is sub divided into nine sub cities called Arada, Bole, Addis ketema, Nefas silk lafto, Kolfe Keraniho, Akaki kaliti, Yeka, Lideta, Kirkos and Gulele sub cities.

Addis Ababa lies at an altitude of 2326 meters from its lowest point around Bole international airport and is a grass land biome located between 9°1'48" North, 38°44'24" East latitude and longitude respectively. The alternating dry and rainy season in the area are almost fixed annually. The long rainy season extends from June to September, contributes about 84% of the total annual rainfall while the dry season lasts from October to February. The short rainy season lasts from March to May, the mean annual minimum and maximum temperature are 14°C and 21°C respectively with all over average 17°C, the mean relative humidity is 61.3% [15].

**Study Population:** Data were collected from 365 lactating dairy cows, out of which 255 lactating Holstein Friesian, 85 Jersey and 25 local Zebu breeds of cows which were kept under intensive production system of the study area. Animals were selected systematically.

**Study Design:** A cross sectional study was conducted to determine the prevalence of mastitis from December 2011 to April 2012 in five sub cities of Addis Ababa at cow and quarter level based on clinical manifestations for clinical mastitis and indirect tests (CMT and culture) for subclinical mastitis. The study was based on the questionnaire survey, physical and clinical examinations of clinical cases, screening tests using CMT for sub clinical cases, aseptic meticulous sampling and microbiological investigation.

### Sample Size Determination and Sampling Strategy:

The sample size was determined at 95% confidence interval, 5% precision and from previous studies in the study area [22], with an expected prevalence of 39%. Thus, the sample size value was calculated based on Thrusfield, [24] and the sample size was 365 animals. Systematic random sampling technique was employed to select the lactating animals that were sampled for CMT test in that selected farm. Before sample collection for bacteriological examination, milk samples were examined for visible abnormalities and were screened. Samples were taken for culturing from strong positive CMT screening test and biochemical tests was done for further bacterial isolation.

**Milk Sample Collection:** Milk sample collection was according to the procedures recommended by National Mastitis Council (NMC) [25] to avoid the effect of time between milking and sampling. The udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with a pledged of cotton moistened (but not completely wet) with 70% ethyl alcohol. Recontamination of teats during scrubbing was avoided by scrubbing carefully, the teats on the far side of the udder first, then those on the near side. Separate pledged cotton was used for each teat. Teats towards sample collection were sampled first and then the far ones. The first 3-4 streams of milk were discarded. The collecting vial was held as near horizontal as possible and by turning the teat to a near horizontal position, approximately 10 mL of milk was collected into a universal sample collection bottle. After collection, the sample was placed in icebox and transported to the laboratory.

**California Mastitis Test screening:** CMT was carried out according to the method described by Quinn *et al.* [16]. A squirt of milk, about 2 mL from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in a horizontal plane for 15 seconds. The result was scored from 1-4 and the interpretation is presented in table 1.

**Bacterial Isolation:** Bacteriology was performed on all quarters milk showed strong positive for CMT. Out of 1460 quarters, 48 were found blocked and two samples were lost during handling, hence, milk samples were collected from 127 strong positive quarters and cultured. Identification of mastitis pathogens was carried out following microbiological procedures for diagnosis of bovine udder infection described in NMC [25]. Milk samples that had been refrigerated, dispersion of bacteria and fat were accomplished by warming the samples at room temperature (25 °C) for about an hour and then mixed by shaking. The samples were allowed to stand for a while for the foam to disperse and just before inoculation the tube was inverted gently. One standard loop (0.01ml) of milk sample was streaked on Plate Count Agar media. The inoculated plate was incubated aerobically at 37 °C. The plates were checked for growth after 24, 48 and up to 72 h to rule out slow growing microorganisms such as *Corynebacterium* species.

For primary identification, colony size, shape, color, Gram staining reaction and catalase production were used. This was conducted at Addis Ababa Regional Laboratory center. For confirmation, biochemical tests were used after sub culturing isolated distinct colony on to a nutrient agar. The procedures followed for the identified pathogens are different for different species of bacteria. Generally the collected samples were inoculated into peptone water (nutrient media) and incubated over night, culture on plate count agar media and incubated for 24 h, then Gram stain was done to differentiate those Gram negatives and Gram positive bacteria. Gram positives were culture on MacConkey agar media and perform different tests like catalase, motility, indole, TSIA, H<sub>2</sub>S and EMB. While Gram negative bacteria was sub cultured on nutrient agar media to perform specific tests like catalase, motility, indole, oxidase, coagulase, CAMP and MSA tests. Interpretation was made according to NMC [25]. The culture was considered negative if no growth occurs after 72 h of incubation. Isolation of two or more colonies from a quarter samples was considered contaminated and the result was discarded.

Table 1: Interpretations for CMT

CMT score	Interpretation	Visible reaction
0	Negative	Milk fluid is normal
1	Trace	Slight precipitation
2	Weak positive	Distinct precipitation but not gel formation
3	Distinct positive	Mixture thickness with gel formation
4	Strong positive	Strong gel that is cohesive with a convex surface

Data collection and analysis: Depending on clinical inspection and CMT results cases were categorized as either positive or negative. Positive cases were further categorized as clinical and subclinical mastitis. Age of the study animals was determined from birth records and categorized as young adults (2-4 years), adults (5-7 years) and old (>8 years). Data related to previous history of the mammary quarters and causes of blindness were obtained from clinical records of the farm and interviews with the owner of the farms. The data were recorded in Microsoft Excel spread sheet for statistical analysis by SPSS version 20 was used. Collected data were summarized using descriptive statistical analysis. The prevalence was calculated by dividing the number of positive cows/quarters by the total number of cows/quarters tested [24].

## RESULTS

**Animal Level Prevalence:** Mastitis prevalence at cow level was 52.6% (192/365), out of which 14.6% (28/192) and 85.4% (164/192) experienced clinical and subclinical, respectively. Samples from all 28 clinical

cases and 97.6% (124/127) of the CMT positive subclinical quarters were found to be culture positive. Therefore a total of 124 (97.6%) culture growth were observed (Table 2).

**Quarter Level Prevalence:** Out of 1412 quarters examined 421 (29.8%) teats were found positive for mastitis in this study. Out of the 1460 quarters 48 had blind teats. The details of the mastitis prevalence at quarter level were shown in Table 3.

**Bacterial Isolation:** From those 127 strongly CMT positive, 124 properly grown bacteria of 7 genera were isolated. The most prevalent mastitis causing pathogen in this study was *Staphylococcus* (42.5%), of which the predominant species were hemolytic, coagulase positive *S. aureus* (24.4%). *Streptococcus* species (26.9%), *E. coli* (11.8%), *Klebsiella* (5.5%), *Salmonella* (4.7%), *Corynebacterium* (3.1%) and *Pasteurella* species (3.1%) were also isolated with decreasing order of frequency as a genus level. The results of various bacterial species isolated from the clinical and subclinical cases are shown in table 4.

Table 2: Prevalence of clinical and subclinical mastitis at cow level and culture results of mastitic cows.

Forms of mastitis	No of positives	Percent (%)	No of cultured	Percent (%)
Clinical	28	7.7	28	28 (100)
Sub clinical	164	44.9	99	96 (97)
Total	192	52.6	127	124 (97.6)

Table 3: Prevalence of mastitis at quarter level

Quarter sampled	Negatives No. (%)	Positives No. (%)	Blind No. (%)
Front left	228 (62.5)	126 (34.5)	11 (3)
Front right	254 (69.6)	101 (27.7)	10 (2.7)
Hind left	259 (71.0)	91 (24.9)	15 (4.1)
Hind right	250 (68.5)	103 (28.2)	12 (3.3)
Total	991 (67.9)	421 (28.8)	48 (3.3)

Table 4: Bacterial species isolated from cows with clinical and sub clinical mastitis

Organism isolated	Number of isolates		Total Number	Proportion
	Clinical	Sub clinical		
<i>E. coli</i>	7	8	15	11.8
<i>Salmonella</i> spp.	0	6	6	4.7
<i>S. aureus</i>	8	22	31	24.4
Coagulase negative <i>Staphylococcus</i> spp.	2	21	23	18.1
<i>S. agalactia</i>	4	22	19	15.0
Other <i>Streptococcus</i> spp.	4	11	15	11.9
<i>Klebsiella</i> spp.	2	5	7	5.5
<i>Corynebacterium</i> spp.	0	4	4	3.1
<i>Pasteurella</i> spp.	1	3	4	3.1

## DISCUSSION

The overall prevalence of mastitis in the current study was 52.6% based on CMT screening. The finding of this study was slightly lower than the previous reports of Bishi [26] and Mekbib *et al.* [27] who reported prevalence rates of 69.8 and 71% respectively in different farms in and around Addis Ababa and Holeta areas. While those were higher than those reported by Biffa *et al.* [28] and Nessru [29] which were 33 and 25% respectively. The variability in the prevalence of bovine mastitis among the reports could be attributed to difference in management system of the farm, breed considered, level of production and differences in study methods. Overall quarter recorded prevalence was 29.8% in the current study which was higher than the findings of Biffa *et al.* [28] and Almaw [30] who reported quarter prevalence of 17.9%. Blind quarters (3.3%) recorded in this study were an indication of serous mastitis problem in this study area. This finding was comparable with the earlier reports in Asella [31] and Bahir Dar area [32].

The present finding showed that prevalence of subclinical mastitis was higher 85.4% (164/192) than clinical mastitis 14.6% (28/192). However, this finding was much higher than the previous findings recorded in different dairy farms in the country [19,22,23,28,29,34,35] who reported 5% clinical and 32.2% sub clinical cases in the urban and peri-urban dairy farms at Addis Ababa, central Ethiopia. In this study as well as in other similar studies, overwhelming cases of mastitis were subclinical compared to clinical mastitis in all breeds [22] [23]. Reports have indicated that in Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases while the high economic loss could come from subclinical mastitis [21]. According to Radostits *et al.* [14], an infected quarter showed 30% and a cow 15% reduction in milk yield. Usually Ethiopian dairy farm owners especially smallholders are not well informed about the invisible loss from subclinical mastitis [36] since dairying is mostly a sideline business in these owners. Earlier report has indicated that subclinical mastitis remains the most economically damaging and zoonotic potential disease for dairy industry [37].

In this study *S. aureus* were the predominant pathogens involved constituting 24.4% of all isolates. The isolation rate of *S. aureus* in the present finding was higher than the findings of Bishi [26] and Hussein [36] who reported 9% and 10.69% prevalence respectively in

Addis Ababa. However, the isolation rate of *S. aureus* found in this study was lower than the findings of Workineh *et al.*, [22] and Kerro and Tareke [23] where *S. aureus* accounted for 39.2 and 40.5% of the isolates, in their study at Addis Ababa and southern Ethiopia respectively. Relatively the higher prevalence of *S. aureus* in this study could be associated with total absence of dry cow therapy and post milking teat dipping, the invariably hand milking practice, low culling rate of chronically infected cows (culling was usually due to feed shortage, aging and reproductive problem) and limited knowledge of farmers on segregation as a control option. The primary reservoir of contagious pathogens including *S. aureus* is infected quarter and the exposure of uninfected quarter is limited to the milking process [38]. The predominance in prevalence of this organism may be associated with its frequent colonization of teats, its ability to exist intracellular and localize within micro abscesses in the udder and hence resistant to antibiotic treatment [39]. This organism is also well adapted to survive in the udder and usually establishes a mild sub clinical infection of long duration from which it shed in milk facilitating transmission to healthy animals mainly during milking [14].

The second most significant isolate recorded in this study was coagulase negative *Staphylococcus* (18.1%) compared to others (*Corynebacterium* species 3.1%, *S. agalactia* 15%, other *Streptococcus* species 11.9%, *Salmonella* (4.7%), *Pasturella* (3.1%). Kerro and Tareke [23], reported isolation of coagulase negative *Staphylococci* at a rate of 2.5% lower than the present study in a prevalence study in southern region and Addis Ababa, Ethiopia. However, the present finding was lower than that of Bishi [26] and Hussein [36] in Ethiopia who reported 54 and 42% prevalence. The high isolation rate of coagulase negative *Staphylococcus* in this study could be associated with lowered resistance of the cow due to teat injury. *Staphylococci* typically colonize a broken skin and hence abrasion of the teat end increases the risk of staphylococcal colonization at the teat end and subsequent transfer into the udder [6].

Streptococcal species causing mastitis recorded in the present study (26.9%) was in agreement with findings of Zerihun [41] (27%) but much lower than the amount reported for the same species by Kingwill *et al.* [42] who recorded 80.95% prevalence rate in dairy cows. The lower isolation rate in this study might be associated with the widespread use of penicillin in the area for treatment of mastitis. It has been recognized that mastitis caused by *Streptococcus* species is susceptible to eradication via

use of penicillin [14]. The relative higher prevalence of *Corynebacterium* mastitis in this study (3.1%) compared with reports of Biffa, [28] (4.55%) and Zerihun [41] (1.9%). Consistent with former report by Radostis *et al.* [14] that higher incidence of *Corynebacterium* spp. is associated with lack of post-milking teat dip.

In conclusion this study showed that bovine mastitis is among the major problems hindering dairy productivity in the study area. The study also revealed that Staphylococcal and Streptococcal species were the major bacterial pathogens that cause of bovine mastitis, especially subclinical mastitis, in intensive dairy farms of Addis Ababa. Therefore, this warrants the development of appropriate of control and prevention measures under the prevailing husbandry system. Awareness on subclinical mastitis to those farm owners should be promoted in order to increase milk production in the study area.

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