JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

BOVINE MASTITIS, ASSOCIATED RISK FACTORS AND ANTIMICROBIAL RESISTANCE PATTERNS OF ISOLATES AROUND DEBRE BERHAN, NORTH CENTRAL HIGHLAND OF ETHIOPIA

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JANUARY 2017

JIMMA, ETHIOPIA

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MSc Thesis

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A thesis Submitted to the School of Graduate Studies of Jimma University College of Agriculture and Veterinary Medicine in the Partial Fulfillment of the Requirements for the Masters Degree in Veterinary Epidemiology

JANUARY 2017

JIMMA, ETHIOPIA

DEDICATION

This thesis manuscript is dedicated to my beloved grandmother, Ayelech Tedebabe for nursing me with affection, discipline and love and her dedicated support for success in my life.

STATEMENT OF AUTHOR

I declare that this thesis is my effortful work that all sources of materials used for this have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirement for MSc degree in Veterinary Epidemiology at Jimma University College of Agriculture and Veterinary medicine, School of Veterinary Medicine and it can then be deposited at the university/ college library for borrowing according to the rule of the library. On the other hand, I solely declare that this thesis is not submitted to any other body anywhere for the award of any academic degree, diploma, or certificate. Quotations from this thesis are allowable with accurate acknowledgement of the source.

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Date of submission: 13/12/2016

BIOGRAPHICAL SKETCH

Kasahun was born in Abichu na Negna district in Mendida town, North Shewa Zone of Oromia National Regional State, in February 1985G.C. He attended kinder garden school at Mendida Catholic kinder garden school, elementary education at Mendida Junior secondary school from 1991/92-1998/99 G.C. and secondary education at Mendida Cistercian Monastery Catholic Secondary School from 1999/2000- 2001 G.C. The author successfully passed the National Organization Examination and followed his preparatory education in Hailemariam Mamo Preparatory Secondary school at Debre Berhan from 2002-2003 G.C. He successfully passed the 'Ethiopian Higher Education Entrance Examination' and joined Jimma University College of Agriculture and Veterinary medicine in 2004G.C. After graduation, he worked in agricultural office, as a field veterinarian, at Janamora district in North Gonder for six months. He worked as a lecturer at ATVET College in Alage for four years. Finally, he transferred to Debere Berhan University and he is now a lecturer in the University. In 2015, he joined again Jimma University College of Agriculture and Veterinary medicine (JUCAVM) to pursue a study for an MSc program in Veterinary Epidemiology

ACKNOWLEDGEMENTS

Foremost, I sincerely thank Debere Berhan University for offering me the MSc opportunity and Jimma University College of Agriculture and Veterinary Medicine for the research funding.

I would like to express my sincere gratitude to my major advisor Prof. Tadele Tolosa for the continuous support of my MSc study and research, for his patience, motivation, material support, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. Besides my major advisor, I would like to thank my co advisor Dr. Yohannes Muluneh, for his encouragement, insightful comments, material support and laboratory work assistance. I would like to thank Dr. Feyissa Begna for his support during my study.

I wish to extend my profound gratitude to Debre Berhan research center, North Shewa Zonal agricultural office, Basona Warana agricultural office, Jimma University College of Agriculture and Veterinary Medicine library and ICT staff and their workers for their full cooperation during my work.

My sincere thank also go to Dr. Kassa Demese for supporting me materially and spiritually. Finally, I must express my very profound gratitude to my parents and to my beloved wife, Alemayehu Abera, for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis.

TABLE OF CONTENTS

Contents Page

DEDICATION XI
STATEMENT OF AUTHOR XII
BIOGRAPHICAL SKETCH XIII
ACKNOWLEDGEMENTS ..... **Error! Bookmark not defined.**
TABLE OF CONTENTS XV
LIST OF FIGURES XVI
LIST OF ANNEXS XVIII
LIST OF ABBREVIATION XIX
ABSTRACT XX
1. INTRODUCTION 1
2. LITERATURE REVIEW 4
2.2. The Disease 4
2.2.1. Aetiology 5
2.1.2. Epidemiology of mastitis 7
2.2.2. Risk Factors 8
2.2.2.Clinical findings of the disease 14
2.2.3. Diagnosis 14
2.2.3.Treatment and control of the disease 16
2.3. Antimicrobial resistance 18
2.4. Epidemiology of Bovine Mastitis in Ethiopia 19
2.4.1. Prevalence of Bovine Mastitis in Ethiopia 19
2.4.2. Bacterial isolates in different dairy farms of Ethiopia 20
2.4.3. Risk Factors of Bovine Mastitis in Ethiopia dairy farms 20
2.5. Antimicrobial sensitivity test in different dairy farms of Ethiopia 22
3. MATERIAL AND METHODS 23
3.4. Study Area 23
3.5. Study Population and Study Animals 24
3.6. Study Design and Sampling Procedure 24
3.7. Detection of Mastitis 25

3.8. Milk Sample Collection and Transportation 25
3.9. Culture Procedure and Bacteriological Isolation 26
3.10. Antimicrobial Susceptibility Testing 26
3.11. Data Analysis 27
4. RESULT ..... **Error! Bookmark not defined.**
4.1. The Overall Prevalence of Mastitis 29
4.2. Risk Factors for of Mastitis 30
4.3. Prevalence of Bacterial Pathogens 31
4.4.*In Vitro* Antimicrobial Susceptibility Test Result 32
5. DISCUSSION 34
6. CONCLUSIONS AND RECOMMENDATIONS 41
ANNEXES 51

LIST OF TABLES

Table 1. Antioxidant systems of mammalian cells 10

Table 2. Summery on cross-sectional study with the purpose of estinating the prevalence, bacterial pathogens and the associated risk factors of bovine mastitis in different dairy farms of Ethiopia 19

Table 3. Prevalence of clinical and sub-clinical mastitis at herd, cow and quarter levels..29

Table 4. Occurrence of clinical mastitis and sub clinical mastitis at front and hind quarters29

Table 5. Occurrence of clinical, subclinical mastitis and blind teat at each quarter....**Error! Bookmark not defined.**

Table 6. CMT result at quarter level.....**Error! Bookmark not defined.**

Table 7. Association of risk factors with mastitis.....30

Table 8. Isolated bacterial and their relative prevalence.....31

Table 9. Kirby Bauer disk diffusion method *in vitro* antimicrobial susceptibility test result. 33

LIST OF FIGURE

Figure 1. Study area 23

LIST OF ABBREVIATIONS

AMC	Amoxicillin
AOR	Adjusted Odd Ratio
CMT	California mastitis test
CNS	Coagulase negative staphylococcus
CPS	Capsular polysaccharide
CSA	Central Statistical Authority
DCT	Dry cow therapy
DNA	Deoxyribonucleic acid
E	Erythromicin
EMA	Ethiopia Metrology Agency
FAO	Food and Agriculture Organization of the United Nations
GDP	Gross Domestic Product
G .C	Gregorian calendar
GEN	Gentamicin
K	Kanamicine
LF	Left front
LR	Left rear
M.a.s.l.	Meter above sea level
Mpcr	Multiplex Polymerase Cain Reaction
NCCLS	National Committee for Clinical Laboratory standard
NMC	National Mastitis Council
OR	Odd Ratio
PCR	Polymerase Chain Reaction
PG	Penicillin G
RF	Right Front
RFLP	Restricted Fragment Length Polymorphism
RR	Right rear
SCC	Somatic Cell Count
Spp	Species
S	Streptomycin
USAID	United state Agency for International Development
VIF	Variance Inflation Factor
WHO	World Health Organization

ABSTRACT

This study was carried out between March 2016 and August 2016 to estimate prevalence of mastitis, identify associated risk factors, to identify and isolate causative bacterial pathogens and to assess their antibiotic sensitivity patterns in smallholder dairy farms at Basona Warana district around Debre Berhan, North Shewa Zone, Ethiopia. Purposive sampling was followed to select the study area, Basona Warana district, based on its dairy potential and infrastructure facilities. Simple random sampling technique was used to select dairy farms. Accordingly, among 1500 smallholder dairy farms in the study area, 187 smallholder dairy farms were selected based on the list of the farmers from the dairy cooperatives of the district. A total of 187 herds were examined, of which 79.7% [95% CI: 73.9% - 87.4%] herds had mastitis, in which 10.7% and69% had clinical and subclinical mastitis, respectively. A total of 403 lactating cross breed lactating cows were selected and examined by physical examination of udder and milk and using California Mastitis Test (CMT). Out of the total examined cows 73.2% [95%CI: 68.2%-78.3%] had mastitis, of which 26% and 67.2% had clinical and subclinical mastitis, respectively. Out of 1612 examined quarters, 47.6% [95%CI: 44-51.2] quarters were mastitis positive, in which 21.7%, 47.6% and were clinical and subclinically positive, respectively. From the total examined quarters 1.1 % quarters [95%CI: 0.6-1.7] were blind teat. Association of bovine mastitis with different risk factors was checked using logistic regression model. The multivariable analysis revealed that the odds of being infected with mastitis were higher in cow with large parity number (OR=1.8) than cows with low parity number. The multivariable analysis also showed the odds of acquiring mastitis was higher in cows those washed every day (OR= 4.9) than cows washed with long gap and the risk of the disease was higher in cows with ≥3 lactating cow per herd (OR= 1.8) than cows with <3 lactating cow per herd. The predominant bacteria isolated were Staphylococcus spp 71.1% followed by Streptococcus spp 23% while E. coli 2.9% was the least isolates. The antimicrobial sensitivity test result showed that most of the isolated bacteria were found to be sensitive to Gentamicine, Kanamayacin, Streptomycin, Erythromycin and Penicillin G. Nevertheless, the isolates were resistant to Amoxicillin. The present study shows subclinical form of mastitis is highly prevalent in the study area. Stage of lactation, frequency of cow washing and number of lactating cow per herd were risk for mastitis. Staphylococcus spp were the most dominant isolates followed by streptococcus spp. Hence, attention should be given at later lactation by using dry cow therapy, proper washing and drying of cows and handling manageable size of herds and appropriate use of antimicrobial drugs could reduce the high prevalence mastitis in the study area.

Keywords: Antimicrobial Sensitivity, Bovine mastitis, North Shewa zone, Prevalence, Risk factors

1. INTRODUCTION

Ethiopian economy depends on agriculture. Agriculture has two subsectors: livestock and crop production. The livestock sector alone contributes about 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the agricultural GDP (Metaferia *et al*., 2011), 15% of export earnings and 30% of agricultural employment (Behnke, 2010).The cattle population in Ethiopia estimated to be about 56.71 million(CSA, 2015). Out of this total cattle population, the female cattle constitute about 55.45 percent. Dairy-cows are estimated around 6.5 million (11.46%) and milking-cows are about 11.4 million (20.07%) heads of the total female cattle population. The population of cross and exotic breeds accounted about 1.19% and 0.14% respectively (CSA, 2015).

The increase in human population, accessibility to technological input and high demand for animal product purchasing power in urban center had helped the urban and per-urban dairy farm in the country to flourish (Yoseph *et al.,* 2000). Even though Ethiopia has huge number of livestock, the productivity has always been sub-optimal due to low genetic potential of the animals, poor nutrition and prevailing diseases (Belayneh *et al*., 2013). Among the diseases, mastitis is one of the most important problems of dairy cattle causing huge economic losses to the dairy industry. Mastitis is an inflammation of the mammary gland and commonly associated with intramammary bacterial infection. Mammary infections are divided into two categories, clinical and sub-clinical. The most important changes in many clinical cases are changes in the milk include discoloration and the presence of clots. There is swelling, heat, pain and edema in the mammary gland (Radostits *et al*., 2007). Sub-clinical mastitis is described as the presence of an infection without visually evident sign of local inflammation or systemic involvement (Erskine, 2011).

The prevalence of bovine mastitis is influenced by a number of different risk factors, which includes animal, environmental and pathogen risk factors. The animal risk factors include age, parity, stage of lactation, morphology and physical condition of udder and teat and breed. The environmental risk factors are quality and management of housing, milking practice and

season of the year. Whereas, the pathogen risk factors are viability of pathogens, virulence factors, colonizing ability and types of toxins of the pathogens (Radostits *et al*., 2007).

A number of pathogens are reported to be the cause of mastitis in dairy cows of which bacterial agents are the most common one (Bradley, 2002). The most commonly incriminated and reported pathogens of mastitis in different parts of Ethiopia with different rate include, *Staphylococcus aureus, Cogulase negative staphylococci, Streptococcus agalactiae, Streptococcus dysagalactia, Streptococcus uberis, Escherichia coli, Pseudomonas aeroginosa, Actinomyces pyogenes, Corynebacterium bovis, Enterococcus fecalis, Klebsiella pneumonia, Bacillus* spp. and *Micrococcus spp*. (Adane *et al.,* 2012*;* Belayneh *et al*., 2013; Duguma *et al*., 2014; Degn *et al.*, 2015; Demeke *et al*., 2016).

In Ethiopia, the prevalence of the mastitis has been reported in different parts of the country. The association of clinical and subclinical forms of the disease with different risk factors was described. Age, stage of lactation, breed, udder hygiene, parity, milking practice, teat injury and history of previous mastitis were some of the risk factors that contribute for the occurrence the disease (Mekibib *et al.,* 2010; Moges *et al.,* 2011; Girma *et al.*, 2012; Zeryehun *et al.*, 2013; Tilahun and Aylate, 2015; Mokonen *et al*., 2016).

Dairy cattle affected with clinical and subclinical mastitis are routinely treated with antimicrobials (Arestrup, 2005). However, inappropriate and over-use of antibiotics to treat microbial infections and consequent antibiotic selection pressure are the major factors contributing for the reduction of strains susceptibility to antibiotics (WHO, 2012). Thus, antibiotic sensitivity test is important to isolate resistant strains of pathogens.

North Shewa Zone is a high potential cereal-livestock area, where dairy activities play a significant role in the livelihood of farmers. It is a potential dairy production area within the Addis Ababa milk-shed. The area is also supplying a considerable volume of milk to government and private milk processing plants. Considering its potential and the economic significance of dairy production to the local community repeated efforts has been performed by governmental and non-governmental aid organizations to improve the dairy production and productivity (Argaw and Tolosa, 2008). However, in the study area, mastitis is the one

causing huge economic loss to the dairy sector as farmers' reports ascertain. The information on the prevalence of mastitis and associated risk factors and causative pathogens in the study area is limited.

Therefore, the main aim of this study was:

- to estimate the prevalence of bovine mastitis, its potential risk factors and causative pathogens in small holder dairy farms around Debre Berhan.

The specific objectives of:

- To estimate the prevalence of clinical and sub clinical mastitis in lactating cows at herd and cow
- To assess the associated risk factors at cow level
- To isolate and identify the predominant causative bacterial pathogens
- To asses antibiotic sensitivity of isolated pathogens

2. LITERATURE REVIEW

2.2. The Disease

Mastitis is inflammation of the parenchyma of the mammary gland, which can result from exposure to a variety of infectious agents. Mammary infections can be divided into two forms, clinical and (sub) clinical. The most important changes in many clinical cases are changes in the milk include discoloration and the presence of clots. There is swelling, heat, pain and edema in the mammary gland (Radostits *et al*., 2007). While, in cases of sub-clinical mastitis are described as the presence of an infection without visually evident sign of local inflammation or systemic involvement (Erskine, 2011). According to Radostits *et al*. (2007) bovine mastitis classified based on causative agent into three types. Which are: -

Contagious mastitis: that is caused by pathogens mostly live inside udders or on teat skin and are spread either by splashes of infected milk or sprays during stripping, on milkers' hands or teat cup liners, and by cross flow of milk between teatcups.

Opportunistic mastitis: that is due to normal teat skin inhabitants and cause mastitis.

Environmental mastitis: is caused by those pathogens usually present in the cow's environment and reach the teat from that source.

Faull and Hughes (1985), classified mastitis based on the degree of inflammation in to:

Normal quarter: is a quarter with no pathogens and few SCC (neutrophils) in the milk and which feels normal.

Subclinical mastitis: is a quarter with pathogens and many SCC (neutrophils) in the milk, but the milk looks normal and the quarter feels normal.

Clinical mastitis: is further classified in to:

Per acute mastitis: is the most serious form of mastitis, which most often endangers the life of the animal. The affected animal shows a very high temperature, remain off feed and show respiratory distress. The udder is swollen and extremely painful. There is cassation of milk secretion and exudates are often blood stained.

Acute mastitis: in this case systemic reactions are slight to moderate. The udder becomes swelled and there is change in the milk.

Sub-acute mastitis: in which variable changes in the milk but practically no changes in the udder tissues. Culture of milk will show presence of pathogenic bacteria.

Chronic mastitis: is the terminal stage of the disease. Udder becomes hard due to fibrosis.

2.2.1. Aetiology

A large number of microorganisms have been reported to cause bovine mastitis. Most of those are bacteria, but fungi and algae may also cause mastitis problems in some herds or regions. The most common udder pathogens are *Staphylococci* (*S. aureus* and several *Coagulase-negative staphylococcal* species (CNS), S*treptococci* (*S. agalactiae*, *S. dysgalactiae*, *S. uberis*) and Coliforms (*E. coli*, *Klebsiella spp*), even though other pathogens, e.g. *M. bovis*, may cause problems in some regions (NMC, 2011). These pathogens have been further classified based on their epidemiology and pathophysiology, as contagious, teat skin opportunistic or environmental mastitis pathogens (Radostits *et al*., 2007).

2.2.1.1. Contagious mastitis pathogens

There are many contagious mastitis pathogens. The most common are *S. aureus* and *S. agalactiae*. *M. bovis* is a less common cause of contagious mastitis; it causes outbreaks of clinical mastitis that do not respond to therapy and are difficult to control. Most outbreaks of *M. bovis* are associated with recent introductions of new animals into the herd (Radostits *et al*. (2007).

2.2.1.2. Teat skin opportunistic mastitis pathogens

They are bacterial pathogens that normally reside on the teat skin. They have the ability to create an intramammary infection via ascending infection through the streak canal. Accordingly, their epidemiology of infections differs from those of contagious and environmental pathogens, and it is useful to consider them in a separate category. *Coagulase-negative staphylococci* are the most common teat skin opportunistic mastitis pathogens (Radostits *et al*., 2007).

2.2.1.3. Environmental mastitis pathogens

Environmental mastitis pathogens are associated with three main groups of pathogens, the *coliforms* particularly *E. coli* and *Klebsiella spp., non-agalacctiae Streptococcus spp.* and *A. pyogenes* (new name *Trueperella pyogenes). Coliform* organisms are a common cause of clinical mastitis; occasionally in a severe peracute form. The most prevalent species are *S. uberis* and *S. dysgalactiae*. *A. pyogenes* is an important seasonal cause of mastitis in dry cows and late pregnant heifers in some parts of the world. Bacterial species associated with bovine mastitis are also categorized in to major and minor pathogens (Rainard and Poutrel, 1988; Radostits *et al*., 2007).

Major pathogens are causes most severe cases of clinical mastitis. Which includes S. *agalactiae, S. aureus, M. bovis, S. uberis, S. dysgalactiae, Streptococcus equinus, Streptococcus bovis, E. coli, Klebsiella spp, Ellterobacter spp. and A. pyogenes.* Minor pathogens are causes of subclinical mastitis and less frequently cause clinical mastitis. They include the *Coagulase negative Staphylococcus spp*. such as *Staphylococcus hyicus* and *Staphylococcus chromogene* (Radostits *et al*., 2007).

Uncommon mastitis pathogens that causes sporadic mastitis and usually affects only one cow or a few cows in the herd, includes *Nocardia asteroides, Nocardia brasiliensis* and *Nocardia jarcinica, Histophilus somni, Pasteurella multocida, Mallnheimia haemolytica, Campylobacter jejuni* and other Gram-negative bacteria including *Citrobacter spp., Leptospira spp., Enterococcus jaecalis, Enterococcus jaecium, Proteus spp,* and *Serratia spp.*

Anaerobic bacteria have been isolated from cases of mastitis, usually in association with other facultative bacteria, e.g. *Peptostreptococcus indolicus, Prcvotella melaninogenica* (formerly *Bacteroides melaninogenicus), Eubacterium combesii, Clostridium sporogenes and Fusobacterium necrophorum.* Fungal infections include *Trichosporon spp., Aspergillus jumigatus, Aspergillus nidulans* and *Pichia spp.*; yeast infections include *Candida spp.,Cryptococcus neojormans, Saccharomyces spp.* and *Torulopsis spp.* Algal infections include *Prototheca trispora* and *Protothcca zopfii* (Radostits *et al*., 2007).

However, some viral diseases like *Pseudocowpox, Herpes Mamillitis, Cowpox, Papilloma, Foot-and-Mouth disease* and *Vesicular Stomatitis* affecting the epithelium of the teat orifice are mentioned to result in or predispose to mastitis (Hillerton *et al*., 2001).

2.1.2. Epidemiology of mastitis

2.2.1.4. Source of Infection

The source is the infected glands of other cows in the herd; however, the hands of milkers can act as a source in contagious pathogens. The exposure of uninfected quarters to environmental pathogens can occur at any time during the life of the cow, including milking time, between milking, during the dry period and prior to first calving in heifers. The source of environmental pathogens is the environment of the cow. Examples include wet bedding, dirty lots, milking wet udders, inadequate pre milking udder and teat preparation, housing systems that allow teat injuries, and poor fly control (Faull and Hughes, 1985).

2.2.1.5. Methods of Transmission

Infection of each mammary gland occurs via the teat canal, the infection originating from either an infected udder or the environment in dairy cattle. The infection originating from infected udders is transmitted to the teat skin of other cows by milking machine liners, milkers' hands, wash cloths and any other material that can act as an inert carrier (Radostits *et al*., 2000).

2.2.2. Risk Factors

Mastitis is a disease influenced by many factors. Microorganisms are responsible for the infection, but for them to enter the mammary glands and establish themselves to the point that they cause an infection, a multitude of factors may be involved (e.g. hygiene, housing, climate, milking machines, feed and genetics) simultaneously. It is even more difficult to generalize about the relative importance of each one, as certain factors affect certain microorganisms.

2.2.1.6. Animal risk factors

Age and parity

The prevalence of infected quarters increases with age, peaking at year 7 (Radostitis *et al.*, *2*000; Radostits *et al*., 2007). This might be due to older cows have largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agent in the cows' udder. However, contradicted result was reported that the prevalence of mastitis was higher in adult cows compared with old cows may be due to bad hygienic condition during calving (Elbably *et al*., 2013). Cows with many calves were at greater risk than those of cows having moderate and few calves. This is probably due to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without a mastitis control program (Elbably *et al*., 2013).

Stage of lactation

Prevalence of clinical mastitis was lower in cow with later lactation. Most new infections occur during the early part of the dry period and in the first 2 months of lactation, especially with the environmental pathogens (Radostits *et al*., 2000). .

Breed

A variety of morphological, physiological and immunological factors contributes to a cow's resistance or susceptibility to mastitis, and each of these factors is influenced to some extent

by heredity. The incidence of mastitis is greater in Holstein-Friesians than in Jerseys, but this may reflect differences in management rather than a true genetic difference. Valid comparisons between breeds have not been reported so far (Radostits *et al*., 2000). Some authors reported no significant difference between different breeds of cow (Rahman *et al.*, 2009; Islam *et al.*, 2010; Hussain *et al.*, 2012).

Morphology of udder and teat

High milking rate and large teat canal diameter have been associated with increased SCC or risk of intramammary infection. Decreasing teat-end-to floor distance is also a risk factor for clinical mastitis and may be associated with an increased incidence of teat lesions. Per parturient udder edema may also be a risk factor for clinical mastitis (Radostits *et al*., 2000). Dimitar and Metodija, (2012) reported that cows with inverted teat end shape have a higher incidence of mastitis than cows with small round and pointed teat end shape but there was no associated with decreasing teat end to floor distance and increased incidence of clinical mastitis. Hussain *et al.* (2012) also reported that the higher prevalence of mastitis was in cattle having round and pendulous udder. It may be due to the reason that long and pendulous udder gets injuries and helps the pathogens to grow.

Physical condition of teat

The teat end is the first barrier against invading pathogens, and the efficiency of teat defense mechanisms depends on the integrity of teat tissue; its impairment leads to an increase in the risk of intramammary infection. Teat thickness is an aid to evaluating teat tissue status. Milking machine characteristics can induce a decrease or increase in teat thickness after milking compared with premilking values. Increases in teat thickness of more than 5% are significantly associated with infection and new infection, but the association was not significant when teat thickness decreased by more than 5 %. *Coagulase-negative staphylococcal* infections are significantly associated with both increases and decreases in teat thickness numerically greater than 5%, but there is no association between teat thickness and *S. aureus* infections (Radostits *et al*., 2000).

Quarters with moderate and very severe hyperkeratosis of the teat-end were at significantly increased risk of clinical *E. coli* mastitis (Breen *et al.*, 2009). Hussain *et al.* (2012) report that different teat and udder lesions udder edema was an important risk factor for mastitis which may impairs milk removal. The use of *oxytocin* may have been linked to the opening of teat orifice which remains open for longer time and the teat muscles remain in relaxed position thus enabling the easy entry of pathogens to the udder eventually leading to mastitis (Hussain *et al.*, 2012).

Udder hygiene

Dirty udders are associated with increased SCC and an increased prevalence of intramammary infection due to contagious pathogens, but surprisingly are not associated with intramammary infections due to environmental pathogens (Breen *et al.*, 2009).

Nutritional status

Vitamin E, vitamin A and selenium may be involved in resistance to certain types of mastitis. Early reports found that supplementation with antioxidants such as selenium and vitamin E had a beneficial effect on udder health in dairy cattle by decreasing the incidence and duration of clinical mastitis. An increase in selenium concentration in whole blood was associated with a decrease in all infections, including S. *aureus, A. pyogenes,* and *S. Bovis* (Radostits *et al*., 2000).

Trace minerals and vitamins that can influence udder health include selenium (Se) and vitamin E, copper, zinc, and Vitamin A and β-carotene.

Table 1. Antioxidant systems of mammalian cells

Component (location in cell)	**Nutrients Involved**	**Function**
Superoxide dismutase (cytosol)	Copper and zinc	An enzyme that converts superoxide to hydrogen peroxide

Superoxidedismutase (mitochondria)	Manganese and zinc	An enzyme that converts superoxide to hydrogen peroxide
Ceruloplasmin	Copper	An antioxidant protein, may prevent copper from participating in oxidation reactions
Glutathione peroxidase (cytosol)	Selenium	An enzyme that converts hydrogen peroxide to water
Catalase (cytosol)	Iron	An enzyme (primarily in liver) that converts hydrogen peroxide to water
α-tocopherol (membranes)	Vitamin E	Breaks fatty acid peroxidation chain reactions
β-carotene (membranes)	β -carotene	Prevents initiation of fatty acid peroxidation chain reactions

Source: National Mastitis Council Annual Meeting Proceedings (2002)

Doherr *et al.* (2007) reported that nutritional and associated metabolic products are risk factors for the occurrence of subclinical mastitis in organic producing farm not in commercial producing farm. That is related with increased milk urea concentrations. Use of mineral supplements was associated with an enhanced risk for subclinical mastitis but there are no obvious explanations why this was only true in organic producing farm but not in commercial producing farm.

Milk yield

High milk yielding cows are generally considered to be more susceptible to intramammary infection because in the high-yielding cows the glandular tissues are more susceptible to infection (Radostits *et al*., 2000; Rahman *et al.*, 2009; Islam *et al.,* 2010; Hussain *et al.*, 2012).

Other concurrent diseases

Retained placentas, teat injuries, infected uterine discharge and teat sores might be associated with a higher incidence of mastitis (Peeler *et al.,* 1994; Radostits *et al*., 2000). Sole ulceration of any severity occurring in more than one digit has been associated with an approximately three fold higher risk of *S. aureus* infections in the first lactation. Cows with a history of mastitis in the preceding lactation are twice as susceptible to clinical mastitis in the current lactation as those without mastitis in the preceding lactation (Radostits *et al*., 2000). Cow gets infected or diseased during the periparturient period and becomes more susceptible to udder infection due to lowered immunity (Nickerson, 1994; Peeler *et al.*, 1994; Rahman *et al.*, 2009). Milk fever is another concurrent disease occurred with mastitis. In milk fever, low level of calcium decreases the rigidity of the teat sphincter that perhaps allows the organism to pass into the udder. Calcium ions are necessary for muscle constriction (Paape and Guidry, 1993).

2.2.1.7. Environmental and management risk factors

Radostits *et al.* (2000) explain quality and management of housing factors such as climate, housing system, type of bedding and rainfall interact to influence the degree of exposure of teat ends to mastitis pathogens. Because dairy cattle spend 40-65% of their time lying down, the quality and management of housing for dairy cattle has a major influence on the types of mastitis pathogen that infect the mammary gland, as well as the degree of infection pressure. Housing lactating cattle on sawdust leads to intramammary infection. Those cows housing on sawdust are infected six times more with *Klebsiella* bacteria and twice as much *coliform* on the teat ends compared to housing cattle on sand. Any housing factor or management system that allows cows to become dirty or damage teats or that causes overcrowding will result in an increase in clinical mastitis. This includes the small size of stalls and alleyways, difficult of movement of cattle, poor cleaning system, overcrowding, poor ventilation, access to dirty ponds of water and muddy areas. Lack of maintenance of strict hygiene and good sanitary environment may be a contributory factor in the cause of mastitis (Rahman *et al.*, 2009; Elbably *et al.*, 2013).

Milking practices

Wet teats and udders are a risk factor for increased SCC, especially in the presence of teat impacts from liner slippage. Increasing person-hours spent milking per cow may be associated with a higher rate of clinical mastitis. Contaminated milking equipment including milk hoses, udder wash towels and teat dip products are associated with outbreaks of environmental mastitis (Radostits *et al*., 2000). The infection usually spread from cow to cow at milking if the milking hygiene is not good enough (Haltia *et al.*, 2006).Use of udder towels for more than one cow was found to be associated with a higher prevalence of contagious pathogens (Plozza *et al.*, 2011).

Season of year

The relationship between the incidence of mastitis and season of the year is variable, depending on geographical and climatic conditions. In subtropical and tropical areas, the incidence is higher during winter or spring calving from the increase in infection pressure which associated with increased humidity. In temperate climates, the incidence of mastitis is higher in autumn and winter, when calving occurs along with an extended period of housing (Radostits *et al*., 2000). Rahaman *et al*. (2009) also reported that the prevalence of mastitis higher in wet than in dry season. Others reported that the frequency of mastitis is higher during summer season followed by winter and spring possibly due to exposure of teats to a dirty environment, and teat lesions resulting from various causes, probably resulted in the increased intramammary infections (Elbably *et al.*, 2013)**.**

2.2.1.8. Pathogen risk factors

Different pathogen risk factors contribute for the occurrence of bovine mastitis. This includes viability of the pathogen, virulence factors, colonization ability and toxins. Potential virulence factors that are produced by mastitis causative pathogens include lipopolysaccharide endotoxin (by *E. coli*), enterotoxins, coagulase, alpha, beta, delta toxins, hemolysin, hyaluronidase and leukocidins (by *S. aureus*) and hyaluronidase and the hyaluronic (by *S. uberis*) (Radostits *et al.*, 2000).

2.2.2. Clinical findings of the disease

The symptoms of bovine mastitis are different based on the type of the disease and the degree of inflammation. Mastitis can be with visible symptom or without visible symptom. Mastitis with visible symptom is called clinical mastitis. The clinical findings include very high temperature, remain off feed, respiratory distress, swollen and extremely painful udder, cassation of milk secretion and exudates are often blood stained. Clinical mastitis can be more manifested by slight to moderate systemic reactions and swollen udder with milk change. At the chronic stage of the disease the adder become hared due to fibrosis (Radostits *et al.*, 2000).The other forms of mastitis is the invisible type of mastitis called subclinical. In this case of mastitis there is no visible symptom on quarter or udder of the cow but an elevation of SCC (neutrophils) in the milk and losses in milk yield. Thus, culture of milk will show presence of pathogenic bacteria (Faull and Hughes, 1985).

2.2.3. Diagnosis

Clinical mastitis is easily recognized by the appearance of abnormal milk, swelling of gland and /or illness. Subclinical mastitis seems normal milk and not recognized by visual observation. Hence, it requires indirect tests to detect such cases.

2.2.1.9. Somatic Cell Count (SCC)

Somatic cell counter is the most commonly used automated device for rapid determination of SCC in milk samples. This instrument stains cells with a fluorescent dye and then counts the number of fluorescing particles (Schalm *et al*., 1971).

SCC is used to detect individual cow and monitor herd problems. Different studies show different threshold to quarter and cow (Dohoo and Meek, 1982; Larsen, 2000; Hamann, 2003). Monitor herd mastitis also has thresholds for milk quality control and these also differ between regions (Emanuelson, 1997; Larsen, 2000). Nevertheless, no information is available on this particular issue in Ethiopia.

2.2.1.10. California Mastitis Test (CMT)

The California Mastitis Test (CMT) remains the only reliable screening test for subclinical mastitis that can be easily used at the cow side (Schalm *et al.*, 1971). The CMT was developed to test milk from individual quarters but also been used on composite and bulk milk samples.

The CMT involves mixing and swirling equal parts of bromocresol violet reagent and milk in a plastic paddle with a compartment for each quarter (Quinn *et al*., 1999).The test results are interpreted subjectively as either 0 (negative), T (trace), +,++ or +++ based on the viscosity of the gel formed by mixing the reagent with the milk (NMC, 1990).

2.2.1.11. Culture

Most mastitis control programs include the use of individual cow cultures to determine which mastitis pathogens are present on the farm. The microbiological examination of both individual cow and bulk tank culture are elements of mastitis control. Culturing can be used in a targeted fashion for specific control programs such as segregation plans for contagious mastitis or for surveillance to detect the presence of new or emerging pathogen. Culturing is also used to evaluate treatment efficacy and to establish susceptibility patterns to aid in the development of rational treatment strategies (Larsen, 2000).

2.2.1.12. Molecular Test of Mastitis

Polymerase chain reaction (PCR) testing for determination mastitis

The use of PCR is very basic to many molecular procedures. It is also important to understand that the PCR copies the sequences of both living and dead bacteria. Identification of bacterial DNA does not ensure that an active bacterial infection is currently present in the mammary gland. When molecular methods are used, producers must understand that there are multiple sources of bacterial DNA and the utility of the samples will be vastly improved when aseptic methods are used to collect the milk samples. Thus, the use of molecular testing for making individual cow decisions is not yet well defined. To facilitate decision making, the medical

history, microbial culture of the milk and SCC of the cow should be combined with the results of the molecular test (Mahmmod *et al*., 2013).

Shome *et al*. (2011) reported that the developed mPCR assay was found to be simple, rapid, reliable and specific in species identification of 10 bacteria at a time. This includes *S. aureus, S. chromogenes, S. epidermidis, S. sciuri, S. haemolyticus, S. simulans, S. agalactiae, S. dysgalactiae, S. uberis and E. coli*.

DNA Fingerprinting

DNA fingerprinting is used to determine individual strains of a bacterial species. There are several different methods for comparing the DNA of a bacterial species, but they are all based on extraction of bacterial DNA followed by separation of the bacterial DNA into columns based on size of specific fragments. Some of the methods include Pulsed Field Gel Electrophoresis (PFGE) or Restricted Fragment Length Polymorphism (RFLP). The resulting bands of DNA fragments are compared to each other and form the basis for deciding if strains are identical, similar or different. Bands that are identical or very similar are considered to be the same strain or a slightly different substrain (Zadoks and Schukken, 2006).

2.2.3. Treatment and control of the disease

2.2.3.1. Treatment

Because of the diverse bacterial etiologies of the disease a variety of control methods involving hygiene prior to, during and after milking are used to minimize exposure of cows to mastitis organisms. Despite these procedures, new cases of mastitis invariably occur and antimicrobial therapy plays a role in the control of bovine mastitis (Owens *et al.*, 1997).

Alekish *et al.* (2013) reported that *S. aureus* and *E.coli* showed higher susceptibility to Enrofloxacin, Ciprofloxacin, Gentamycin, and neomycin. *S. agalactiae* and *S. uberis* were complete susceptibility to different anitbiotics, except *S. agalactiae* was resistant to Lincomycin and Streptomycin and *S. uberis* to Cloxcillin and Streptomycin (Idriss *et al.*, 2014).

2.2.3.2. Control

Mastitis is a complex disease, and there is no simple solution for its control. So understanding its occurrence, the related risk factors, and the mastitogenic pathogens involved are fundamental elements in developing a control program (FAO, 2014).

The NMC (national mastitis control council) has developed a 10- points mastitis control program for udder health such as, maintain a clean environment, proper milking procedures, maintain and use milking equipment properly, manage clinical mastitis during lactation, good records keeping, dry cow management program, follow a biosecurity program, monitoring udder health status and periodically review mastitis control program (http://www.nmconline.org/).

Efforts have been made to develop a vaccine against mastitis, but neither satisfactory outcome have been claimed in the field nor on backyard farms (Poutrel *et al*., 1988; Leitner *et al*., 2000; Leitner *et al*., 2003; Buzzola *et al*., 2006; Nour *et al*., 2006; Chang *et al*., 2008). It is clear that a single vaccine will not prevent mastitis because of the plethora of pathogens and their different mechanisms of pathogenesis (Heath, 2011).

Vaccine against *S. aureus* developed using varied approaches. These include whole organism vaccines (Leitner *et al*., 2003), DNA vaccine encoding clumping factor A (El-Din *et al*., 2006), live attenuated (aroA) *S. aureus* (Buzzola *et al*., 2006), capsular polysaccharide (CPS)-protein conjugate vaccines (Poutrel *et al*., 1988; Leitner *et al*., 2000) and recombinant *S.aureus* mutated enterotoxin type C (Chang *et al*., 2008).

A new vaccine, *Startvac (Hipra),* has recently been made available in the market targeting not only coliforms but also *coagulase-negative staphylococci* and *S. aureus.* J-5Bacterin, also known as the *E. coli* J5 vaccine, is composed of the J5 mutant strain of *E. coli.* However, no vaccines are available in the market against mastitis caused by *K. pneumonia* (González *et al*., 1989; Hogan *et al*., 1992).

2.3. Antimicrobial resistance

Antibiotics are used extensively in the dairy industry to combat disease and to improve animal performance. Antibiotics such as Penicillin, Cephalosporin, Streptomycin and Tetracycline are used for the treatment and prevention of diseases affecting dairy cows caused by a variety of gram-positive and gram-negative bacteria. Antibiotics are often administrated routinely to entire herds to prevent mastitis during the dry period. An increase in the incidence of disease in a herd generally results in increased use of antimicrobials, which in turn increases the potential for antibiotic residues in milk and the potential for increased bacterial resistance to antimicrobials (Oliver and Murinda, 2012). In many instances, there are improper uses of antimicrobial agents in treatment of mastitis. A single strain may predominate because of the antimicrobial resistance, host adaptation or other factors (Leptolainem *et al*., 2003).

Antimicrobial resistance has been detected in *S. aureus* isolates collected from intra mammary infection at frequencies which vary widely by compound and region sampled. *S. aureus* which is major pathogen of mastitis are resistance to Penicillin or Ampicillin because of the long term use of B-lactam antibiotics in agricultural and health care settings (Kang *et al*., 2007). The *S. aureus* isolates tested were resistance to penicillin and Ampicillin, but that resistance to other compounds such as Tetracycline, Erythromycin and Oxacillin was low, ranging from 8.5% down to less than 1% (Erskine *et al.*, 2002).

Antimicrobial susceptibility determined in vitro has been considered as a prerequisite for treatment. However, activity in vitro does not guarantee efficacy *in vivo* when treating bovine mastitis. Antimicrobial resistance amongst mastitis pathogens has not yet emerged as a clinically relevant issue, but geographical regions may differ in this respect. The biggest problem is the widespread resistance of staphylococci, particularly *S. aureus*, to penicillin G (Hendriksen *et al*., 2008).

2.4. Epidemiology of Bovine Mastitis in Ethiopia

2.4.1. Prevalence of Bovine Mastitis in Ethiopia

A number of studies have been conducted on bovine mastitis in different dairy farms of Ethiopia earlier (Argaw and Tolosa, 2008; Lakew *et al*., 2009; Tesfaye *et al.*, 2012; Duguma *et al*., 2014; Biressaw *et al*., 2015; Belina *et al*., 2016).

Table 2. Summery on cross-sectional study with the purpose of estimating the prevalence, bacterial pathogens and the associated risk factors of bovine mastitis in different dairy farms of Ethiopia

Location	**No**	**Over all prevalence (%)**	**Clinical (%)**	**Sub clinical (%)**	**Authors**
Addis Ababa	2681	40.1	1.2	38.9	Kassa *et al*.(1999)
Repi and Debre- Zeit	186	59.7	21.5	38.2	Workineh *et al.*(2002)
Sellale	500	24.1	1.8	22.3	Getahun *et al*. (2008)
Bahar Dar	302	28.2	3	25.2	Bitew *et al*. (2010)
Holeta	107	71	22.4	48.6	Mikibib *et al*. (2010)
Batu	278	56.5	5.3	40.6	Duro and Taddele, (2011)
Gondar	322	32.6	0.93	31.67	Moges *et al.* (2011)
Doba	384	23.2	7.3	15.9	Girma *et al*. (2012)
Hawassa	183	35.5	4.9	30.6	Moges *et al.* (2012)
Addis Ababa	300	65.3	22	42.3	Tadesse and Chanie, (2012)
Adama	206	48	6.3	41.7	Tesfaye *et al.* (2012)
Asella	66	66.6	12.1	54.5	Abera *et al*. (2013)
Adama	303	39.5	5.9	33.6	Belayhune *et al.* (2013)
Holleta	90	81.1	7.8	73.3	Duguma *et al.* (2014)
Areka	384	52.9	9.4	43.5	G/Michael *et al*.(2013)
Wolaita Sodo	349	29.5	2.6	26.9	Yohannis and Molla, (2013)
Addis Ababa	499	74.7	19.6	51.1	Zeryehun *et al*. (2013)
Gambella	121	60.33	11.57	48.76	Deng *et al.* (2015)
Addis Ababa	444	68	21.2	46.8	Tilahun and Aylate, (2015)
North Shewa	144	88.9	8.3	80.6	Hailemeskel *et al*. (2014)
Arsi	156	42	5.3	36.7	Biressaw and Tesfaye, (2015)
Dire Dawa	385	53.3	9.1	44.2	Tsegaye *et al*. (2015)
Kombolcha	150	56	10	46	Tassew *et al.* (2016)

2.4.2. Bacterial isolates in different dairy farms of Ethiopia

Variation exists on the type and isolation rate of mastitis pathogens from place to place; the most commonly incriminated and reported causes of mastitis in Ethiopia include *S. aureus, Cogulase negative staphylococci, S. agalactiae, S. dysagalactia, S. uberis, E.coli, P. aeroginosa, A. pyogenes, C. bovis, E. fecalis, K. pneumonia, Bacillus* spp. and *Micrococcus* spp. (Sori *et al*., 2005; Lakew *et al*., 2009; Adane *et al., 2012;* Girma *et al*., 2012; Belayneh *et al*., 2013; G/Michael *et al*., 2013; Yohannis, and Molla, 2013; Zeryehun *et al.* ,2013; Duguma *et al*., 2014; Zenebe *et al*., 2014).

S. aureus were the most frequently isolated pathogens followed by *aesculin-positive cocci, S. agalactiae, S. dysgalactiae, S. uberis, Klebsiella spp., E.coli* and *P. aeruginosa* (Tolosa *et al*., 2015).

Table 3. Summery on major isolated bacteria in different dairy farms of Ethiopia

Location	**Isolated bacteria**						
	S. aureus	**CNS**	***S. agalactia***	***S. dysgalactia***	***S. uberis***	***E. coli***	**Authors**
Sebeta	44.9	14.9	3.7	4.5	3	0.8	Sori *et al*. (2005)
Selalle	41.5	2	13.7	2.9	9.8	0.5	Getahun *et al*. (2008)
Asella	24.1	17.3	12.9	6.8	3.8	7.5	Lakew *et al*. (2009)
Bahar Dar	20.3	51.9	8.8	5.1	2.5	2.5	Bitew *et al*. (2010)
Ambo	27.6	21.4	12.2	6.1	3	6.1	Araga *et al.* (2012)
Doba	35.5	-	19.9	5.8	5.8	5.8	Girma *et al*. (2012)
Adama	32.2	23	10.4	3.3	6	3.3	Belayhune *et al.* (2013)
Areka	54.4	4.4	1.6	24.8	5.2	0.4	G/Michael *et al*.(2013)
Wolaita Sodo	30	13.3	17.8	8.9	-	17.8	Yohannis and Molla, (2013)
Holleta	43.3	3.9	12.2	7.2	2.8	-	Duguma *et al.* (2014)

2.4.3. Risk Factors of Bovine Mastitis in Ethiopia dairy farms

Different researchers reported various risk factors for the occurrence of bovine mastitis in different dairy farms of Ethiopia. Age, stage of lactation, breed, udder hygiene, parity, milking practice, teat injury and history of previous mastitis are factors that contribute for the occurrence of bovine mastitis in Ethiopia dairy farms (Mekibib *et al.*, 2010; Arga *et al.*, 2012; Girma *et al.*, 2012; Zeryehun *et al.*, 2013; Tilahun and Aylate, 2015).

2.4.3.1. Age and parity

Girma *et al.* (2012) reported that the prevalence of mastitis was higher in animals older than 12 years followed by animals in the age range of 8 to 12 years and lowest in animals younger than 8 years. The prevalence of mastitis rose with an increase in parity number (Lakew *et al.,* 2009; Bitew *et al.,* 2010; Tesfaye *et al.,* 2012; Zerehun *et al.,* 2013).

2.4.3.2. Lactation stage

Different studies were also reported that early lactation stage had higher relative prevalence than late and mid lactation stage (Bitew *et al.,* 2010; Belayneh *et al.,* 2013; Zeryehun *et al.,* 2013). However, Girma *et al.* (2012) reported cows are at higher risk of acquiring mastitis when they are in early lactation stage and it was found to decrease as lactation stage increased.

2.4.3.3. Breed

Bitew *et al.* (2010) reported that Fogra breeds were genetically controlled physical barriers like streak canal sphincter muscle, keratin in the teat canal or shape of teat where pointed teat end. In addition to physical barriers could arise from differences in occurrence of mastitis in cellular immunity. Lakew *et al.* (2009) reported that the prevalence of mastitis was higher in Holstein-zebu crosses than in indigenous Arsi breeds. Contradicted result reported that blood level had no effect on the incidence rates of mastitis in cross bred cows (Arga *et al.,* 2012).

2.4.3.4. Milking practice

Cows managed under poor hygienic condition had risk of contracting the disease than those managed in good hygienic condition and owners who didn't use towel before and after milking found to have high prevalence of mastitis than owners who used towel (Lakew *et al.,* 2009; Mekibib *et al.,* 2010; Girma *et al.,* 2012; Zeryehun *et al.,* 2013). Tilahun and Aylate, (2015) reported that manual milking methods was the major predisposing factors to increase the prevalence of mastitis.

2.4.3.5. Previous mastitis history and teat injury

Different results reported that the occurrence of mastitis was significantly higher in cow with in moderately tick infestation, teat injury and previous mastitis history (Lakew *et al.,* 2009; Mekibib *et al.,* 2010; Belayneh *et al.,* 2013; Degn *et al.,* 2015).

2.4.3.6. Type of management

Reports indicated that the incidence rate of mastitis was higher in cows kept under the intensive management system compared to cows under the semi-intensive management system (Arga *et al.,* 2012). This could be due to the restricted exercise of animals and the transmissions of pathogens from infected cows to other animals were housed together.

2.5. Antimicrobial sensitivity test in different dairy farms of Ethiopia

Different antibiotics are using in Ethiopia for the treatment of bovine mastitis. Among those antibiotic Gentamycin and Amoxicillin drugs are most widely used in many parts of Ethiopia. They are sometimes the only available antibiotics in many veterinary clinics. It might be this wide use of these drugs and inappropriate administration which have contributed to the development of resistance by the predominant bacterial agents (Girma *et al*., 2012).

Various results shows that the antimicrobial sensitivity test of most milk bacterial isolates including the major pathogens had multiple but variable resistance pattern (Getahun *et al*., 2008; Girma *et al*., 2012; Abera *et al*., 2013; Belayneh *et al*., 2013). Among the isolated pathogens, *S. aureus* and *S. epidermidis* were more susceptible to Kanamycin and Erythromycin and showed strong resistance to Polymixin B (Getahun *et al*., 2008).

Girma *et al*. (2012) reported that among *in vitro* disc sensitivity tested antibiotics Cloxacillin is the most effective drug followed by Gentamycin and Amoxicillin in the study area. While other results reported that Gentamaycine, Chloroamphenicol and Kanamaycine were the most effective antibiotics (Belayneh *et al*., 2013; Abera *et al*., 2013).

3. MATERIAL AND METHODS

3.1. Study Area

The study was conducted around Debre Berhan at Basona worana districts of North Showa Zone of Amhara region. Debre Berhan, the capital town of North Shewa zone, located at 130km North East of Addis Ababa, the capital of Ethiopia at an elevation of 2,840m.a.s.l. Agro-climatically, the zone is divided into Dega (37.4%), Woina Dega (30.1%) and Kola (32.5%).Most parts of the district is characterized by cold temperature varies from 6 to 20°C (MOA, 1998) and is categorized into Highland (*Dega)* (>2,300 m.a.s.l) with bimodal rainfall. The short rainy seasons are running from March to April whereas the long rainy seasons are from June to September. The average annual rainfall ranges from 731 to 1 068 mm. The study area is known by its high potential cereal-livestock production system where dairy activities play a significant role in the livelihood of farmers. According to CSA (2015) report, the annual milk production of North Shewa Zone is about 37,219,833 litters. Zonal agricultural office reported there are 14 milk cooperatives in the Zone, of which 6 found in Angolalla Tera, 3 found in Debre Berhan and 5 found in Basona Woran.

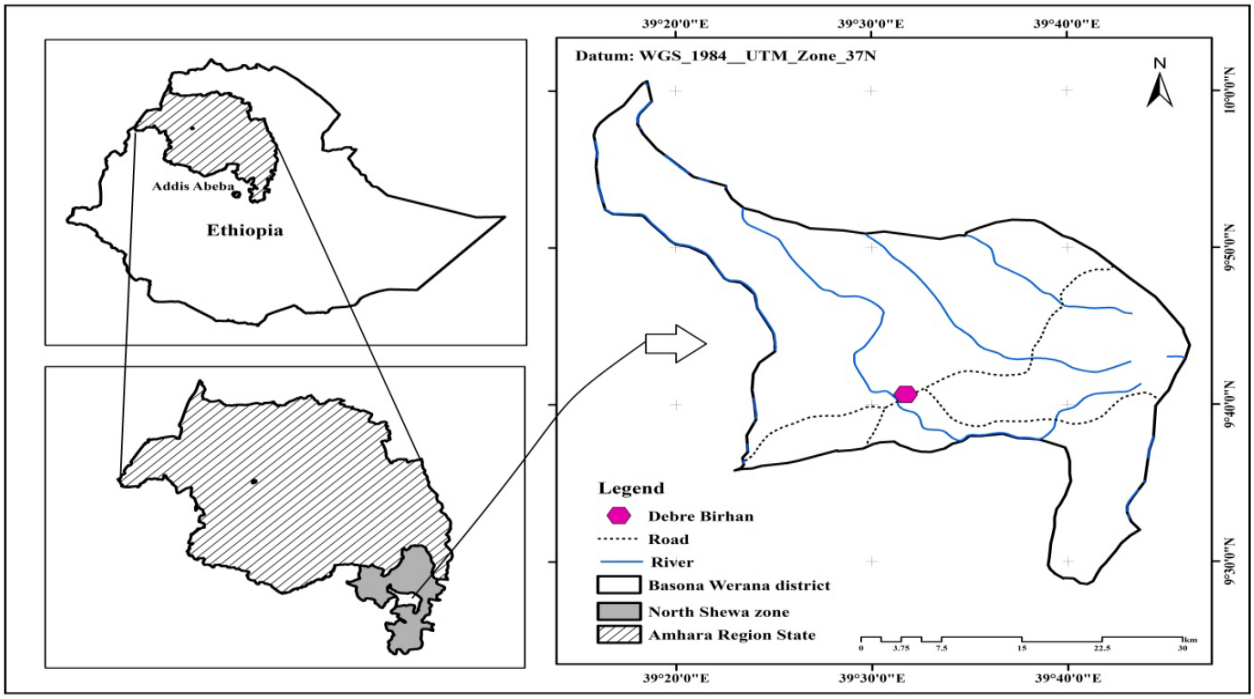


Figure 1. Map of the study area

3.2. Study Population

The total cattle population of the Zone is estimated to be 1,392,619; of which female accounts about 514,097. The Zone has good dairy potential with 16,480 dairy cow and 166,465 milking cows. The study district, Basona worana has total of 186,136 cattle population. Out of which 6,593 (3.5%) heads of cattle are cross breeds (CSA, 2015). Dairy cows in the study area are kept under semi intensive management system. The study population is all smallholder dairy farms in the Basona warana district. The study animal was all lactating cross breed cows of selected smallholder dairy farms of Basona worana district.

3.3. Study Design, Sampling Procedure and Sample size

A cross-sectional study was carried out between March 2016 and August 2016. The study area, Basona Warana was selected purposively based on its dairy potential and infrastructure facilities. Simple random sampling was carried out to select smallholder dairy farms based on the list of the farmers from the dairy cooperatives of the district. Out of 1,500 small holder dairy farms 187 smallholder dairy farms were selected. Cluster sampling method was carried out to select lactating cows. After selection of the farms all lactating, cows in the selected farms were sampled as a study animal. There are two lactating cows on average in each smallholder dairy farms. Finally, a total of 403 lactating cross breed cows were sampled.

The study was conducted through farm inspection, questionnaire survey, animal examination and laboratory investigation. Relevant information was included in the questionnaire, which were intrinsic factors and extrinsic factors. Of intrinsic factors, parity and stage of lactation were included in the questionnaire. Whereas, the management factors were type of housing system, flooring type, bedding type, frequency of cleaning the herd, frequency of washing the whole body of cow, concentrate feeding, milking mastitis positive cow last, premilking udder preparation, use of udder drying towel, use of separate drying towel, washing hand before milking, post milking teat dipping, frequency of milking, awareness about sub clinical mastitis, type of milking, feeding after milking, number of lactating cow per herd, cow hygiene and tick lesion included in the questioner. The selected smallholder dairy farms were visited once (when the questionnaire survey, clinical examination and milk sampling were done in one visit).

3.4. Detection of Mastitis

Gross abnormalities of the udder like the presence of swelling, pain, hotness, disproportional symmetry, fibrosis, visible injury, tick infestation, atrophy and teat blindness was detected by physical examination and were indicative of clinical mastitis. It was also recognized based on abnormalities in milk including flakes, clots and watery secretion (Quinn *et al*., 2004 and Radostitis *et al*., 2007). Cows were considered positive for clinical mastitis, when at least one quarter turned out to be positive for clinical mastitis. A herd was considered positive for clinical mastitis, when at least one cow in a herd was clinical positive (NMC, 1990).

California mastitis test (CMT) was carried out to screen sub-clinical mastitis and for selection of samples for bacterial culture. A small amount of milk from each quarter was squired into shallow cups in the CMT paddle, an equal amount of 3% CMT reagent was added to each cup and mixed well. A gentle circular motion was applied to a mixture in a horizontal plane for 15 seconds. The test result was interpreted based on the thickness of the gel formed by CMT reagent and milk mixture and scored as negative (0), Trace (T), + (weak positive), ++ (distinctive positive), and +++ (strong positive). Quarters with CMT score of trace (T) or above were judged as positive. Cows were considered positive for CMT, when at least one quarter turned out to be positive for CMT. A herd was considered positive for CMT, when at least one cow in a herd was tested positive with CMT (NMC, 1990).

3.5. Milk Sample Collection and Transportation

In order to prevent contamination with the many microorganisms present on the skin of cow's flanks, udder and teats, on the hands of the sampler, and in the barn environment, a strict aseptic procedures was followed during milk samples collection (Quinn *et al*., 1994). After sample collection, sample containing bottles were labeled and transported in an icebox to Debre Berhan University microbiology laboratory. Upon arrival, the samples were stored in a refrigerator at 4°C until analyzed.

3.6. Bacteriological examination of milk sample

California mastitis test (CMT) and clinically positive samples were analyzed microbiologically using procedures described by Quinn *et al.* (1999). A loop full of milk sample was inoculated into blood agar and MacConkey agar plates and inoculated aerobically at 37°C for 24 to 48 hours. Identification of the bacteria on blood agar plate was done based on colony morphology, haemolysis (type of haemolysis, presence or absence of haemolysis) and staining technique. The colonies were sub-cultured on selective media such as, manitol salt agar base for differentiation of *Staphylococcus*. Conventional biochemical tests, catalase and coagulase test, were used to differentiate pathogenic bacteria. Catalase test was used to differentiate between catalase-positive *staphylococci* and *catalase-negative* s*treptococci.* Coagulase test was used to distinguish *S. aureus* from *coagulase negative staphylococci*. *S. agalactiae* was differentiated from other mastisis causing s*treptococci* by using CAMP test. Gram-negative isolates grown on MacConkey agar were identified based on colony characteristics and indole test (Quinn *et al*., 1994; Quinn *et al*., 1999).

3.7. Antimicrobial Susceptibility Testing

Isolated pathogens were tested for 6 commercially available antimicrobials using the Kirby-Bauer disk diffusion method (NCCLS, 2002; Quinn *et al*., 2004). The following antimicrobial discs with their corresponding concentration were used for testing (Himedia Laboratories Put.Limited Mumbai, India): Amoxicillin (30µg/disc), Erythromycin (15µg/disc), Gentamicin (10µg/disc), Kanamicin (30µg/disc), Streptomycin (10µg/disc) and Penicillin G (10µg/disc). Colonies isolated from pure culture were transferred into sterile test tube of 5ml saline and suspension was made. The turbidity of the suspension was adjusted by using sterile saline or adding more isolated colonies to obtain turbidity visually comparable with that of 0.5 McFarland standards. Muller-Hilton Agar plate was prepared, and a sterile cotton swab was dipped in to the suspensions and swabbed on the whole surface of Muller-Hilton Agar plate. The antimicrobial discs were applied on to the surface of the inoculated agar plates using sterile forceps aseptically and pressed gently to ensure the complete contact with the agar surface. The discs were deposited with centers at least 24 millimeter apart. The plates were read 24 hrs after incubation at 37^{o}c under aerobic condition (Quinn *et al*., 2004). The isolates

were classified in accordance with the guideline of the National Committee for Clinical Laboratory Standards (NCCLS, 2002) as susceptible, intermediate or resistant for each antimicrobial tested according to the manufacturer's instructions by measuring the diameter of the zone of inhibition around the antibiotic disc by using ruler.

3.8. Data Analysis

The data collected during the study periods were entered into MS-Excel spread sheet and analyzed using SPSS version 20 software. Over all prevalence of bovine mastitis related to specific risk factors was determined as the proportion of affected cows out of the total examined. Whereas, the prevalence of quarter-level bovine mastitis was determined as the proportion of affected quarter(s) out of the total examined quarters (Thrusfield, 2005). The overall prevalence of bovine mastitis at cow level was considered as dependent variables. The intrinsic and extrinsic factors were considered as independent variables. Of intrinsic factors, parity and stage of lactation were included in the questionnaire. Stage of lactation was classified into three (the beginning of lactation- the first three months of lactation period, middle of lactation - the next five to seven months period and end of lactation -the last month of lactation > 7 month). Whereas, the extrinsic factors were type of housing system(indoor and outdoor), flooring type (soil, concert, stone and wood), bedding type (straw, sawdust, shavings wood, sand and no bedding), frequency of cleaning the herd (once a day, twice a day, three times a day and other), frequency of washing the whole body of cow (every day, every week, 15-30 days, >30 days and not washed), concentrate feeding (yes or no), milking mastitis positive cow last (yes or no), premilking udder preparation (yes or no), use of udder drying towel (yes or no), use of separate drying towel (yes or no), washing hand before milking (yes or no), post milking teat dipping (yes or no), frequency of milking (once a day, twice a day and three times a day), awareness about sub clinical mastitis (yes or no), type of milking (Striping or Squeezing), feeding after milking (yes or no), number of lactating cow per herd (1-3 and >3), presence of tick lesion (yes or no) and cow hygiene (poor or good) included in the questionnaire. Based on dairy cow hygiene score card chart a cow was considered to have good hygiene, if the cow was score 1and 2 and poor hygiene if the cow scored 3 and 4. The proportion of antibiotic sensitivity test was determined as the proportion of sensitive, intermediate or resistant isolates out of the total tested individual bacteria species

isolated. While, the cow and management factors were considered as independent variables. The associations between dependent and independent variables were tested by logistic regression model. And also multicollinearity among independent variables was checked by tolerance or VIF. Those risk factors with $p< 0.25$ during univariate analyses were fitted in a multivariable model. For all the analysis performed, $p<0.05$ was taken as statistically significant (Snedecor and Cochran, 1989).

4. RESULT

4.1. The Overall Prevalence of Mastitis

Table 4. Prevalence of clinical and sub-clinical mastitis at herd, cow and quarter levels

Observation level	Clinical mastitis		Sub clinical mastitis		Total	CI
	No tested	No +ve (%)	No CMT tested	CMT +ve (%)	No +ve (%)	
Herd	187	20(10.7)	187	129(69)	149(79.7)	74% - 87.5%
Cow	403	24(6)	403	271(67.2)	295(73.2)	68.9 -77.5%
Quarter	1612	28(1.7)	1566	719(45.9)	747(47.6)	44% - 51.2%

Note: +ve=positive, CI= confidence interval, CMT=California Mastitis Test and No =number;

From the total examined quarters 1.1% quarters [95% CI: 0.6-1.6], belonging to 16 cows had blind teat. From all examined front quarters 44.3% [95% CI: 38.6% - 49%] quarters were mastitis positive and 51% [95% CI: 46.1% - 56%] hind quarters were mastitis positive (Table 5).

Table 5. Occurrence of clinical mastitis and sub clinical mastitis at front and hind quarters

Quarters	Clinical mastitis	No quarters examined with CMT	Sub clinical mastitis	Total	CI
Front	11(1.4%)	789	339(42.9%)	350(44.3%)	38.6% - 49%
Hind	17(2.1%)	777	380(48.9%)	397(51%)	46.1% - 56%
Total	**28(1.7%)**	**1566**	**719(45.9%)**	**747(47.6%)**	**44% - 51.2%**

4.2. Risk Factors for Prevalence of Mastitis

Table 6**.** Risk factors associated with the occurrence of bovine mastitis

Factor and category				**Mastitis**			
		No.	**No. +ve(%)**	**Univariate analyses**		**Multivariable analyses**	
				P-value	**OR**	***P-value***	**OR(95% CI)**
Intrinsic factors	**Category**						
Parity	**≥3**	237	162(68.4)	**0.009**	1.866	**0.013**	1.817(1.133-2.915)
	1-3	166	133(80.1)				
Lactation stage	>240days/ late	111	72(64.9)	**0.049**	1.711	0.060	1.675(0.978-2.869)
	121-240days/mid	134	103(76.9)	0.926	0.974		
	120days/early	158	120(75.9)				
Extrinsic factors							
Housing system	In door	65	48(73.8)	0.898	0.961		
	Out door	338	247(73.1)				
Flooring system	Soil	120	95(79.2)	0.169	0.822	0.597	0.745(0.252-2.200)
	Stone	250	175(70)	0.496	1.339	0.882	1.083(0.380-3.085)
	Concert	33	25(75.8)				
Bedding type	No bedding	309	219(70.9)	0.058	1.735	0.831	1.089(0.499-2.374)
	Straw	94	76(80.9)				
Frequency of cleaning house per day	Once	229	115(67.7)	0.131	1.711	0.324	0.628-4.083
	Twice	119	97(81.5)	0.607	0.813	0.887	0.389-2.264
	Three times	55	43(78.2)				
Frequency of washing cow	Every day	110	88(80)	**0.047**	1.787	**0.003**	4.960(1.723-14.038)
	15-30 days	68	47(69.1)	0.378	0.500	0.425	1.260(0.084-2.147)
	Every week	18	16(88.9)	0.101	1.750	0.442	1.260(0.608-2.268)
	>30 days	207	144(69.6)				
Feeding concentrate	No	105	80(76.2)	0.422	0.809		
	Yes	298	215(72.1)				
Milking order	No	351	255(72.6)	0.517	1.255		
	Yes	52	40(76.9)				
Pre milking udder preparation	No	255	182(71.4)	0.277	1.295		
	Yes	148	113(76.4)				
Use of udder drying towel	No	284	199(70.1)	**0.030**	1.783	0.408	1.671(0.496-5.636)
	Yes	119	96(80.7)				
Use of separate udder drying towel	No	328	239(72.9)	0.752	1.098		
	Yes	75	56(74.7)				
Washing hand before milking	No	227	164(72.2)	0.648	1.110		
	Yes	176	130(74.3)				
Frequency of milking per day	Twice	394	288(73.1)	0.754	1.288		
	Once	9	7(77.8)				
Feeding after milking	No	270	189(70)	**0.040**	1.683	0.264	1.552(0.717-3.360)
	Yes	133	160(79.7)				
Cow hygiene	Poor	255	162(63.5)	**0.025**	1.733	0.428	1.797(0.576-3.670)
	Good	148	133(89.9)				
Udder/teat tick lesion	Yes	97	75(77.3)	0.294	0.750		
	No	306	220(71.9)				
Number of lactating cow per herd	≥3	278	194(69.8)	**0.022**	1.822	**0.043**	1.797(1.020-3.366)
	<3	125	101(80.8)				

Note: +ve= Positive, OR= Odd Ratio, No = number

Cows ≥ 3 parity number had more udder infection (OR=1.8; 95% CI = 1.1-2.9; *P = 0.013*) than cows with < 3 parity number. Frequency of cow washing and number of lactating cow per herd are found to be a risk for mastitis. The risk of the disease was more likely occur in cows those washed every day (OR= 4.9; 95% CI= 1.7-14; *P= 0.003*) than cows washed with long gap. Herds with ≥ 3 lactating cows were more likely to be affected with mastitis (OR=1.8; 95% CI= 1 - 3.2; *P= 0.043*) than herds with < 3 lactating cows (Table 6).

4.3. Prevalence of Bacterial Pathogens

In the present study, a total of 104 quarters milk samples were taken for bacteriological examination. Out of the total samples, 18 samples were from clinical positive quarters and 86 samples were from CMT positive quarters and different bacterial species were isolated. Among isolated bacterial species, *S. aureus* 49% [95%CI: 39.4-58.6] and CNS 22.1% [95%CI: 14.1-30.1] and followed by *Streptococcus* spp (Table 7).

Table 7. Isolated bacterial and their relative prevalence

Type of bacteria isolates	**Clinical**	**Subclinical**	**Total**	**%**	**CI**
S. aureus	11	40	51	49	[39.39-58.61]
CNS	8	15	23	22.1	[14.13-30.07]
S. agalactia	4	8	12	11.5	[5.37-17.63]
S. dysgalactia	2	5	7	6.7	[1.86-11.51]
S. uberis	-	5	5	4.8	[1.39-8.61]
E. coli	3	-	3	2.9	[0.33-6.13]
Mixed growth pathogen	3	-	3	2.9	[0.33-6.13]
Total	**28**	**86**	**104**	**100**	

Note: CI= Confidence interval, CNS= *Coagulase negative staphylococcus*

4.1. *In Vitro* Antimicrobial Susceptibility Test Result

In vitro antimicrobial sensitivity test was done for all isolates and the results of antimicrobial sensitivity tests are presented in Table 8. *S. aureus* were sensitive to Kanamayacin (84%), Erythromycin (82%), Gentamicine (75%) and Streptomycin (69%). However, they were resistant to Amoxicillin (63%) and Penicillin G (53%). CNS isolate were highly sensitive to Erythromycin (91%), Kanamayacin (88%), Gentamycine (87%), Streptomycin (87%) and Penicillin G (65%) and highly resistant to Amoxicillin (70%). The present study also indicated that *S. agalactiae* were completely susceptible to Erythromycin and Kanamayacin (100% to each) and highly sensitive to Gentamycine (83%), Streptomycin (83%) and Penicillin G (67%). But the isolates were resistant to Amoxicillin (75%). *S. dysgalactiae* have been found to show a complete sensitivity to Erythromycin, Gentamycine, Kanamayacin, Penicillin G and Streptomycin the (100 % to each) but resistant to Amoxicillin (84%). *S. uberis* were completely sensitive to Erythromycin, Gentamycine, Kanamayacin and Streptomycin (100%to each) and highly sensitive to Penicillin G (80%). However, they were resistant to Amoxicillin (60%). *E. coli* was completely sensitive to Gentamycine and Streptomycin (100% each) and also highly sensitive to Erythromyci and Kanamayacin (67% each), but resistant to Amoxicillin (100%) and Penicillin G (67%).

In this study, most isolated pathogens are sensitive to Gentamicine (91%), Streptomycin (89%), Kanamayacin (88%), Penicillin G (74%) and Erythromycin (66%) but most of isolated pathogens are resistant to Amoxicillin (76%).

Table 8. Kirby Bauer disk diffusion method *in vitro* antimicrobial susceptibility test result

		AMC30µg			E15µg			GEN10µg			K30µg			PG10µg			S10µg		
Bacteria isolated	**No isolated**	**R %**	**I %**	**S %**	**R %**	**I %**	**S %**	**R %**	**I %**	**S %**	**R %**	**I %**	**S %**	**R %**	**I %**	**S %**	**R %**	**I %**	**S %**
S. aureus	51	63	18	19	2	16	82	-	25	75	-	16	84	53	8	39	22	9	69
CNS	23	70	-	30	-	9	91	-	13	87	-	22	88	26	9	65	-	13	87
S. agalactia	12	75	25	-	-	-	100	-	17	83	-	-	100	-	33	67	-	17	83
S. dysgalactia	7	86	-	14	-	-	100	-	-	100	-	-	100	-	-	100	-	-	100
S. uberis	5	60	-	40	-	-	100	-	-	100	-	-	100	-	20	80	-	-	100
E. coli	3	100	-	-	33	-	67	-	-	100		33	67	67	-	33	-	-	100
Total	**104**	**76**	**7**	**17**	**0.3**	**4.2**	**65.7**	**0**	**9**	**91**	**0**	**12**	**88**	**14**		**74**	**4**	**7**	**89**

Note: S: Susceptible, I: intermediate, R: resistance, AMC: Amoxicillin, E: Erythromicin, GEN: Gentamycine, K: Kanamayacin, PG: Pencilline G, S: Streptomycin.

5. DISCUSSION

5.1. The Overall Prevalence of Mastitis

The overall prevalence of mastitis at herd level was comparable with the previous reports (Getahun *et al.*, 2008; Belayneh *et al*., 2013) but higher than the report of Tesfaye *et al*. (2012) in Adama. The cow level prevalence of mastitis in this study is concurring with the reports of Mekbib *et al.* (2010) and Zerihune *et al*. (2013). However, it is higher than the finding of Biressaw and Tesfaye, (2015) and Belina *et al.* (2016) in Arsi and in North Showa, respectively. The discrepancy in the prevalence of mastitis between various reports could probably be due to differences in farm management practices, breed, geographic location and label of production (Radostitis *et al*., 2007).

The study revealed that the prevalence of clinical mastitis at cow level is similar with the finding of Belayneh *et al*. (2013), Biressaw and Tesfaye (2015) and Demeke *et al*. (2016), who reported 5.9% in Adama town, 5.3 in Arsi and 7% in and around Zeway, respectively. The present result is higher than the report of Getahun *et al*. (2008) in Selalle and Moges *et al*. (2011) around Gonder. However, it is lower than the findings of Zerihune *et al.* (2013) and Tilahun and Aylate (2015) in and around Addis Ababa. The difference between reports may be due to concurrent disease involvement, interaction of several risk factors relating with animal and virulence of causative organism (Radostits *et al*., 2007).

The prevalence of sub clinical mastitis at cow level is in line with the report of Duguma *et al*. (2014) in Hollata. While, it is higher than the report of Dang *et al*. (2015) and Belina *et al*. (2016), who reported 48.8% in Gambella and 40.7% in North Showa respectively. Argaw and Tolosa (2008); Hailemeskel *et al*. (2014) reported higher prevalence of sub clinical mastitis at cow level than the present study.

In this study sub clinical mastitis has been found to be higher than clinical mastitis. A similar observation of the dominance of subclinical mastitis was observed by several studies Duguma *et al*., 2014; Hailemeskel *et al*., 2014; Degn *et al.*, 2015; Demeke *et al*., 2016; Belina *et al*., 2016). This could be due to little attention of the farmers about subclinical mastitis, as the infected animal shows no obvious clinical symptoms and secrets apparently normal milk.

Therefore farmers are not well informed about invisible loss from subclinical mastitis (Belayneh *et al*., 2013; Tassew *et al*., 2016). Therefore, none of the farms screened their cows for subclinical mastitis except seeking professional assistance at the time of clinical case.

The present study shows the hind quarters (51%) are more affected with mastitis than the front quarters (44.3%). This might be due to the high production capacity of the hind quarters and as a result the hindquarter is more prone surface and the pressure on the teat canal forces the canals to be opened widely and highly predisposed for contamination with dirt which allows entrance of microbes (Radostitis *et al*., 2000; Tassew *et al*., 2016).

5.2. Risk Factors Associated With the Prevalence of Mastitis

The present result revealed that the increase the prevalence of mastitis with increase parity number, this was in agreement with previous reports (Moges *et al.,* 2011; Girma *et al*., 2012; Belayneh *et al*., 2013; Biressaw and Tesfaye, 2015; Degn *et al*., 2015; Mekonnin *et al.,* 2016; Tassew *et al*., 2016). It could be due to repeated parturition also exposes cows to environmental and contagious bacteria. Repeated parturition may induce stress and ultimately down regulates the cow's immunity. In general, the immunity of animals decreases through age making older animals more prone to mastitis (Girma *et al*., 2012). It could be due to older cows have largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agent in the cows' udder (Radostitis *et al.,* 2007). In addition, it increases opportunity of infection with time and the prolonged duration of infection, especially in a herd without a mastitis control program (Elbably *et al*., 2013).

In the present study, every day washed cows had high prevalence of mastitis than those cows washed after a week and above. If the skin of the udder is contaminated and then washed and not dried, the water running toward the teat end, it will transport bacteria. Then the bacteria may enter the teat canal. Washing the udder without drying-out later may actually increase the number of bacteria reaching the teat end instead of decreasing it. Inadequately dry the body and udder will increase the chance of entrance of pathogens in to the teat canal (NMC, 2013). The use of a common wash rag or sponge could also be a risk factor for contagious mastitis (Radostitis *et al.,* 2007).

The present study revealed that the prevalence of mastitis increase with increases the number of lactating cow in the herd. Since, as the number of animal in the herd increases, manure disposal and sanitation problems increase, humidity and temperature also increased. High humidity and high ambient temperatures favor growth of pathogens. As a result, exposure to environmental pathogens increased (Radostitis *et al*., 2007). In addition, the exposure of lactating cows to poor environmental condition can induce stress, which reduces the cow's resistance to environmental pathogen infections.

5.3. Prevalence of Bacterial Pathogens

In the present study, *Staphylococcus* is dominant bacterial isolates. *S. aureus* is dominant spp. This result is in line with Mekbib *et al*. (2010) and Garedew *et al*. (2015). However, it is higher than Lakew *et al*. (2009); Bitew *et al*. (2010); Zerihune *et al.* (2013). But, lower than the report of Tassew *et al.* (2016) in Kombolcha.

The high prevalence of *S. aureus* may be due to the presence organism on the skin of the teats and external orifices, bedding materials, feedstuffs, housing materials, non bovine animals in the farm and equipment. Thus, the organism can easily transmit between cows and invade the udder or teat during unhygienic milking practice. The organism has the ability to colonize the epithelium of the teat and the teat canal, and can adhere and bind to epithelial cells of the mammary gland. In addition its ability to exit intracellulary and localize with in micro abscesses in the udder and hence resistance to antibiotic treatments. Furthermore, the organism produces enzymes like coagulase and Leukocidin. These two enzymes provide adequate mechanisms for tissue invention and inactivate inflammatory cells (neutrophils) respectively (Radostits *et al*., 2007). In areas, where hand milking and improper use of drug is practiced to treat mastitis case, its dominance has been expected (Duguma *et al.,* 2014).

The other most prevalent isolate species of *Staphylococcus* are CNS with the rate of 22.1%. This finding is comparable with the report of Araga *et al*. (2012); Belayneh *et al*. (2013) but, higher than Duguma *et al.* (2014) and lower than the finding of Bitew *et al*. (2010) and Hailemeskel *et al*. (2014). *Coagulase-negative staphylococci* are opportunistic pathogens on teat skin that cause mastitis by ascending infection via the streak canal (Radostits *et al*., 2007)

In the present study, *Streptococcus* was the second prevalent bacterial genus isolated and *S. agalactiae* was dominant isolates of all *Streptococcus* spp. This finding is comparable with the findings of Araga *et al.* (2012) and Duguma *et al.* (2014). However, it is higher than the report of G/Michael *et al.* (2013) and lower than the findings of Biressaw and Tesfaye, (2015) and Zerihune *et al.* (2013). *S. dysgalactia* was the second *Streptococcus* spp isolated which is in line with the finding of Lakew *et al*. (2009); Araga *et al.* (2012); and Duguma *et al.* (2014). *S. uberis* is the other *streptococcus* spp isolated which is in agreement with the findings of Lakew *et al*. (2009) and Girma *et al.* (2012). The differences in isolation rate of *Streptococcus* may result from management system and ecological difference in agents (Abera *et al*., 2013). The other reason might be due to study methods, instruments and laboratory techniques employed by investigators. Radostits *et al.* (1994) stated that *Streptococcus* spp. is the most prevalent along with *Staphylococcus* spp. However, the lower prevalence as compared to *Staphylococcus* spp. is because *Streptococcus agalactiae* survives poorly outside the udder, and established infections are eliminated by frequent use of penicillin and other antibiotics.

The *E. coli* reported in this study is comparable with the previous finding (Bitew *et al*., 2010), but higher than report of Getahun *et al*. (2008) in Sellale and G/Michael *et al.* (2013) in and around Areka town. Yohannis and Molla, (2013); Biressaw and Tesfaye, (2015); Mekonnin *et al,* (2016) reported higher isolation rate than the present finding. Environmental pathogens, *E. coli* and *S. uberis*, are normally found in cow feces, and once the bedding (sawdust or other) becomes heavily soiled with cow manure; coliform numbers in the bedding will increase (Radostits *et al*., 2007). The chance of udder infection with coliform organisms will increase with increase contact with soil, manure and contaminated bedding.

5.1. *In Vitro* Antimicrobial Susceptibility Test Result

In this study, *S. aureus* were found to be sensitive to Kanamayacin (84%), Erythromycin (82%), Gentamicine (75%) and Streptomycin (69%). However, they were resistant to Amoxicillin (63%) and Penicillin G (53%). This finding is in close agreement with the findings of Belayneh *et al.* (2013) who reported *S. aureus* was completely susceptible to Gentamicine (100%) and highly susceptible to Kanamayacin (90%) and Streptomycin (54%), but resistant to Amoxicillin (62%) and Penicillin G (53%). Similar study in East Showa Zone

indicated that *S. aureus* was found 75% resistant to Amoxicillin and 89% to Penicillin G (Belayneh *et al.,* 2014). Chandrasekaran *et al.* (2015) in India reported *S. aureus* was highly resistant to Amoxicillin (62%) and Penicillin G (64%), which is in agreement with the present finding. The present finding disagrees with the report in Nitra, Slovakia by Indriss *et al.* (2014) who reported *S. aureus* was highly susceptible to Amoxicillin (79%) and Penicillin G (87%). The other contradictory finding was reported by Sylejmani *et al.* (2015), who reported *S. aureus* susceptible to Amoxicillin (91%) and resistant to Streptomycin (75%). The difference between the reports might be due to the variation in reputational use of the same antibiotic treatment and differences among specific techniques used. The other reason for the resistance of *S. aureus to* Amoxicillin and Penicillin G is probably due to *S. aureus* are often beta-lactamase producers, the enzyme conferring resistance to beta -lactam antimicrobial agents such as Penicillin G and Amoxicillin (Radostitis *et al.,* 2007).

The present study revealed that CNS was highly sensitive to Erythromycin (91%), Kanamayacin (88%), Gentamycine (87%), Streptomycin (87%) and Penicillin G (65%), but highly resistant to Amoxicillin (70%). This report is in agreement with the finding in Slovakia in which CNS was 87% susceptible to Streptomycin and 86% Penicillin G (Indriss *et al.*, 2014). However, it is disagrees with the report of Indriss *et al.* (2014) and Sylejmani *et al.* (2015) who reported CNS was highly susceptible to Amoxicillin 87% and 83%, respectively. Belayneh *et al.* (2013) in Adama dairy farms reported CNS was resistant to Kanamayacin (72%) and Penicillin G (50%), this finding disagrees with the present finding in which CNS was susceptible to Kanamayacin (88%) and Penicillin G (65%).

S. agalactiea in this study showed 100, 100, 83, 83 and 67% susceptibility to Erythromycin, Kanamayacin, Gentamycine, Streptomycin and Penicillin G, respectively. However, the isolates were resistance to Amoxicillin (75%). This finding is comparable with the finding of Getahun *et al*. (2008), who reported *S. agalactiea* were susceptible to Erythromycin (93%), Streptomycin (85%) and Penicillin G (85%). Belayneh *et al.* (2013) indicated that *S. agalactiae* were completely resistant to Amoxicillin (100%) and susceptible to Gentamycine (100%), Penicillin (80%) and Streptomycin (52%) this is in agreement with the present finding. The other findings reported by Indriss *et al.* (2014) and Sylejmani *et al.* (2015) *S.*

agalactiae were susceptible to Amoxicillin (100 and 94%), respectively; this reports disagree with the present finding in which *S. agalactiae* were resistant to Amoxicillin (75%). In the present study *S. agalactiea* was highly sensitive to all antibiotics except Amoxicillin.

S. dysgalactiae have been found be completly sensitivity to Erythromycin, Gentamycine, Kanamayacin, Penicillin G and Streptomycin (100 % to each) but resistant to Amoxicillin (84%). This finding is in agreement with the finding of Getahun *et al*. (2008) and Belayneh *et al.* (2013) who reported *S. dysgalactiae* were completely susceptible to Gentamycine, Penicillin G and Streptomycin (100 % to each). However, the present finding disagrees with the report of Sylejmani *et al.* (2015) in Kosovo who reported *S. dysgalactiae* is highly resistant Penicillin G (75%) and Streptomycin (75%) and completely susceptible to Amoxicillin (100%). The difference in the sensitivity of *S. dysgalactiae* to Amoxicillin and Penicillin G could be due to the two antibiotics the most commonly available and affordable antibiotics to farmers under Ethiopia condition. They are sometimes the only available antibiotics in many veterinary clinics (Girma *et al*, 2012).

The present finding shows *S. uberis* were completely sensitive to Erythromycin, Gentamycine, Kanamayacin and Streptomycin (100% to each) and highly sensitive to Penicillin G (80%), but resistant to Amoxicillin (60%). This finding is in agreement with Getahun *et al*. (2008) and Indriss *et al.* (2014) who reported *S. uberis* were completely sensitive to Erythromycin (100%). According to Belayneh *et al.* (2013) *S. uberis* were highly susceptible to Gentamycine(89%), Kanamayacin (68%), Streptomycin (93%) and Penicillin G (65%) and resistant to Amoxicillin (65%), which is comparable with the present finding. However, the present finding disagrees with the finding of Indriss *et al.* (2014) and Sylejmani *et al.* (2015) who reported *S. uberis* were highly susceptible to Amoxicillin 100 and 86%, respectively.

The study also revealed that *E. coli* was completely sensitive to Gentamycine and Streptomycin (100% each) and also highly sensitive to Erythromyci and Kanamayacin (67% each), but resistant to Amoxicillin (100%) and Penicillin G (67%). This finding is comparable with the finding of Belayneh *et al.* (2013) who reported *E. coli* was highly susceptible to Gentamycine(92%), Kanamayacin (75%) and Streptomycin (65%) and highly resistant to

Amoxicillin (75%) and Penicillin G (79%). The present finding also in agreement with Indriss *et al.* (2014) who reported *E. coli* was highly resistant to Amoxicillin (82%) and Penicillin G (96%). However, Sylejmani *et al.* (2015) reported *E. coli* was found 80% susceptible to Amoxicillin and 73 % resistant to Streptomycin.

In the present study most of isolated pathogens were susceptible to Gentamicine, Kanamayacin, Streptomycin, and Erythromycin, this could be due to these drugs were the least frequently used in the study area in Veterinary services. However, the all isolates were resistance Amoxicillin to and some of them are resistant to Penicillin G, this is due to the two antibiotics the most commonly available and affordable antibiotics to farmers under Ethiopia condition. They are sometimes the only available antibiotics in many veterinary clinics. It might be this wide use of these drugs and inappropriate administration which have contributed to the development of resistance by the predominant bacterial agents in the area (Girma *et al*, 2012).

6. CONCLUSIONS AND RECOMMENDATIONS

The present study revealed that mastitis is prevalent disease in smallholder dairy farms in the study area. Moreover, subclinical mastitis is the most prevalent form of the disease at herd- and cow-level. The hindquarters are the more affected with mammary gland infection than front quarters. Parity, frequent washing of cow and increasing number of lactating cow per herd are important risk factors for the prevalence of the disease in the study area. The study shows that *S. aureus* and *Coagulase negative Staphylococcus* are the most important causes of bovine mastitis followed by *Streptococcus spp* in the study area. This indicates contagious mastitis is highly prevalent in the study area. In the present finding, most isolated bacteria are sensitive to Gentamicine, Kanamayacin, Streptomycin, Erythromycin and Penicillin G. However, they were resistant to Amoxicillin.

Based on the above concluding remarks the following recommendations are forwarded:

- Properly washing and drying of cows should be practice
- Handling manageable size of herds
- Attention should be given at later lactation by using dry cow therapy
- Apply regular antimicrobial sensitivity testing to select effective antibiotics for treatments of mastitis cases.
- Appropriate use of antimicrobial drugs could reduce the high prevalence mastitis in the study area.

7. REFERENCES

Aarestrup, F.M., 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Basic & clinical pharmacology & toxicology*, **96(4)**: 271-281.

Abera, B., Lemma, D. and Iticha, I., 2013. Study of bovine mastitis in asella government dairy farm of Oromia Regional state, South Eastern Ethiopia. *International journal of current research and academic review*, **1(2):**134-135.

Alekish, M.O., Al-Qudah, K.M. and Al-Saleh, A., 2013. Prevalence of antimicrobial resistance among bacterial pathogens isolated from bovine mastitis in northern Jordan. *Rev Med Vet*, **164:**319-326.

Arga, S., Tadesse, G., Tessema, T.S. and Zewdu, E., 2012. Bacterial pathogens and udder infection dynamics during the early lactation period in primiparous cows in Ambro Town, Central Ethiopia. *Global Veterinaria*, **8(4):**403-408.

Argaw, K. and Tolosa, T., 2008. Prevalence of sub clinical mastitis in small holder dairy farms in Selale, North Shewa Zone, Central Ethiopia. *Internet J Vet Med*, **5(1)**.

Bedane, A., Kasim, G., Yohannis, T., Habtamu, T., Asseged, B. and Demelash, B., 2012. Study on prevalence and risk factors of bovine mastitis in Borana Pastoral and agro-pastoral settings of Yabello District, Borana Zone, Southern Ethiopia. *J. Agric. & Environ. Sci*, **12(10):**1274-1281.

Behnke, R., 2010. The contribution of livestock to the economies of IGAD member states: study findings, application of the methodology in Ethiopia and recommendations for further work.

Belayneh, R., Belihu, K. and Tesfaye, A., 2014. Microbiological study on bacterial causes of bovine mastitis and its antibiotics suscebtibility patterns in East Showa Zone, Akaki District, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **6(4):**116-122.

Belayneh, R., Belihu, K. and Wubete, A., 2013. Dairy cows mastitis survey in Adama Town, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **5(10):**281-287.

Belina D., Muktar Y., Hiko A., Tamerat N., Kebede T., Wondimu T. & Kemal J., 201. Belina *et al*., 2016 Prevalence, Isolation of Bacteria and Risk Factors of Mastitis of Dairy Cattle in Selected Zones of Oromia Regional States, Ethiopia. *Global Journals Inc*. **16 (1):**2249-4618.

Biressaw S. and Deme T., 2015. Prevalence of bovine mastitis and determinant of risk factors in lemu Bilbilo District, Arsi Zone. *Global Journal of Veterinary Medicine and Research,* **3 (2):**080-085

Bitew, M., Tafere, A. and Tolosa, T., 2010. Study on bovine mastitis in dairy farms of Bahir Dar and its environs. *Journal of Animal and Veterinary Advances*, **9(23):**2912-2917.

Bradley, A.J., 2002. Bovine mastitis: an evolving disease. *The veterinary journal*, **164(2):**116-128.

Breen, J.E., Green, M.J. and Bradley, A.J., 2009. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. *Journal of dairy Science*, **92(6):**2551-2561.

Buzzola, F.R., Barbagelata, M.S., Caccuri, R.L. and Sordelli, D.O., 2006. Attenuation and persistence of and ability to induce protective immunity to a Staphylococcus aureus aroA mutant in mice. *Infection and immunity*, **74(6):**3498-3506.

Chandrasekaran, D., Nambi, A.P., Thirunavukkarasu, P.S., Venkatesan, P., Tirumurugaan, K.G. and Vairamuthu, S., 2015. Incidence of resistant mastitis in dairy cows in Tamil Nadu, India. *Journal of Applied and Natural Science*, **7(1):**304-308.

Chang, B.S., San Moon, J., Kang, H.M., Kim, Y.I., Lee, H.K., Kim, J.D., Lee, B.S., Koo, H.C. and Park, Y.H., 2008. Protective effects of recombinant staphylococcal enterotoxin type C mutant vaccine against experimental bovine infection by a strain of Staphylococcus aureus isolated from subclinical mastitis in dairy cattle. *Vaccine*, **26(17):**2081-2091.

Council, N.M., 1999. Laboratory handbook on bovine mastitis. NMC Inc., Madison.

CSA (Central Statistical Agency of Ethiopia) (2015). Report on livestock and livestock characteristics; Volume II, Agriculture Sample Survey.

Deng, Y., 2015. Assessment of Hygienic Milk Production and Prevalence of Mastitis in Dairy Cows in Jikawo Woreda of Nuer Zone, Gambella Region, Ethiopia. *Sciences*, **2(2):**480-486.

Dimitar, N. and Metodija, T., 2012. Udder quarter risk factors associated with prevalence bovine clinical mastitis. *Mac Vet Rev*, **35:**55-64.

Doherr, M.G., Roesch, M., Schaeren, W., Schallibaum, M. and Blum, J.W., 2007. Risk factors associated with subclinical mastitis in dairy cows on Swiss organic and conventional production system farms. *Veterinary Medicine- praha-*, **52(11):**487.

Dohoo, I.R. and Meek, A.H., 1982. Somatic cell counts in bovine milk. *The Canadian Veterinary Journal*, **23(4):**119.

Duguma, A., Tolosa, T. and Yohannes, A., 2014. Prevalence of clinical and sub-clinical mastitis on cross bred dairy cows at Holleta Agricultural Research Center, Central Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **6(1):**13-17.

Elbably, M.A., Emeash, H.H. and Asmaa, N.M., 2013. Risk factors associated with mastitis occurrence in dairy herds in Benisuef, Egypt. *World's Veterinary Journal*, **3(1):**5-10.

El-Din, A.N.N., Shkreta, L., Talbot, B.G., Diarra, M.S. and Lacasse, P., 2006. DNA immunization of dairy cows with the clumping factor A of Staphylococcus aureus. *Vaccine*, **24(12):**1997-2006.

Emanuelson, U., 1997. Use of individual somatic cell count in monitoring herd status. *Livestock Prod. Sci*, **48:**239-246.

Erskine, R. J. 2011. Mastitis in cattle. The Merck Veterinary Manual. Merck Sharp and Dohme Corp. Whitehouse Station, N.J.

Erskine, R.J., Walker, R.D., Bolin, C.A., Bartlett, P.C. and White, D.G., 2002. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. *Journal of Dairy Science*, **85(5):**1111-1118.

FAO (Food and Agriculture Organization of the United Nations).2014. Impact of mastitis in small scale dairy production systems. *Animal Production and Health Working Paper*. No. 13. Rome.

Faull, W.B. and Hughes, J.W., 1985. Mastitis notes for the dairy Practioners. Deptt. *Vet. Sci., The University of Liverpool*:54.

G/Michael, L., Deressa, B., Begna, F. and Mekuria, A. (2013). Study on prevalence of bovine mastitis in lactating cows and associated risk factors in and around Areka town, Southern of Ethiopia.*Afr. J. Microbiol. Res.* **7(43):**5051 -5056.

Garedew, L., Melese, B. and Tesfaye, R., 2015. Staphylococcus aureus in mastitic crossbreed cows and its associated risk factors in Addis Ababa City, *Ethiopia. Ethiopian Veterinary Journal,* **19(1):**107-116.

Getahun, K., Kelay, B., Bekana, M. and Lobago, F., 2008. Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. *Tropical Animal Health and Production*, **40(4):**261-268.

Girma, S., Mammo, A., Bogele, K., Sori, T., Tadesse, F. and Jibat, T., 2012. Study on prevalence of bovine mastitis and its major causative agents in West Harerghe zone, Doba district, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **4(8):**116-123.

Gonzalez, R.N., Cullor, J.S., Jasper, D.E., Farver, T.B., Bushnell, R.B. and Oliver, M.N., 1989. Prevention of clinical coliform mastitis in dairy cows by a mutant Escherichia coli vaccine. *Canadian Journal of Veterinary Research*, **53(3):**301.

Hailemeskel D., Admasu P. and Alemu, F., 2014. Prevalence and Identification of Bacterial Pathogens Causing Bovine Mastitis from Crossbred of Dairy Cows in North Showa Zone of Ethiopia. *Global Veterinaria* **13 (2):** 189-195.

Haltia, L., Honkanen-Buzalski, T., Spiridonova, I., Olkonen, A. and Myllys, V., 2006. A study of bovine mastitis, milking procedures and management practices on 25 Estonian dairy herds. *Acta Veterinaria Scandinavica*, **48(1):**1.

Hamann, J., 2003. Definition of the physiological cell count threshold based on changes in milk composition. *Bulletin of the International Dairy Federation*, **381:**9-12.

Heath, P.T., 2011. An update on vaccination against group B streptococcus. *Expert review of vaccines*, **10(5):**685-694.

Hendriksen, R.S., Mevius, D.J., Schroeter, A., Teale, C., Meunier, D., Butaye, P., Franco, A., Utinane, A., Amado, A., Moreno, M. and Greko, C., 2008. Prevalence of antimicrobial resistance among bacterial pathogens isolated from cattle in different European countries: 2002–2004. *Acta Veterinaria Scandinavica*, **50(1):**1.

Hillerton, J. E., Morgan, W. F., Farnsworth, R., Neijenhuis, B. F., Mein, G. A., Ohnstad, I., Reinemam, D. J., Timms, L. (2001): Evaluation of bovine teat condition in commercial dairy herds: infectious and infections. Proceedings of the 2nd International Symposium on mastitis and milk quality held at Compton, United Kingdom: 352-356.

Hogan, J.S., Weiss, W.P., Todhunter, D.A., Smith, K.L. and Schoenberger, P.S., 1992. Bovine neutrophil responses to parenteral vitamin E. *Journal of dairy science*, **75(2):**399-405.

Hussain, R., Khan, A., Javed, M.T. and Rizvi, F., 2012. Possible risk factors associated with mastitis in indigenous cattle in Punjab, Pakistan. Pak. *Vet. J*, **32(4):**605-608.

Idriss, S.E., Foltys, V., Tancin, V., Kirchnerova, K., Tancinova, D. and Zaujec, K., 2014. Mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Nitra, Slovakia. *Slovak Journal of Animal Science*, **47(1):**33-38.

Iraguha, B., Hamudikuwanda, H. and Mushonga, B., 2015. Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. *Journal of the South African Veterinary Association,* **86(1):**1-6.

Islam, M.A., Rahman, A.K.M.A., Rony, S.A. and Islam, M.S., 2012. Prevalence and risk factors of mastitis in lactating dairy cows at Baghabari milk shed area of Sirajganj. *Bangladesh Journal of Veterinary Medicine,* **8(2):**157-162.

Kassa, T., Wirtu, G. and Tegegne, A., 1999. Survey of mastitis in dairy herds in the Ethiopian central highlands. SINET: *Ethiopian Journal of Science,* **22(2):**291-301.

Kerro, O. (1997): A study on bovine mastitis in some selected areas of southern Ethiopia. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, DVM Thesis.

Lakew, M., Tolosa, T. and Tigre, W., 2009. Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. *Tropical animal health and production,* **41(7):**1525-1530.

Larsen, D., 2000. Milk quality and mastitis. *Vet. Microbiol,* **71:**89-101.

Lehtolainen, T., Shwimmer, A., Shpigel, N.Y., Honkanen-Buzalski, T. and Pyörälä, S., 2003. In vitro antimicrobial susceptibility of Escherichia coli isolates from clinical bovine mastitis in Finland and Israel. *Journal of dairy science,* **86(12):**3927-3932.

Leitner G, Lubachevsky E, Glikman E, Winkler M, Saran A., 2003. Development of a Staphylococcus aureus vaccine against mastitis in dairy cows. I. Challenge trials. *Vet Immunol Immunopathol* **93:** 31-38.

Leitner, G., Yadlin, B., Glickman, A., Chaffer, M. and Saran, A., 2000. Systemic and local immune response of cows to intramammary infection with Staphylococcus aureus. *Research in veterinary science,* **69(2):**181-184.

Lema, M., Kassa, T. and Tegegne, A., 2001. Clinically manifested major health problems of crossbred dairy herds in urban and periurban production systems in the central highlands of Ethiopia. *Tropical Animal Health and Production,* **33(2):**85-93.

Mahmmod, Y.S., Klaas, I.C., Nielsen, S.S., Katholm, J. and Toft, N., 2013. Effect of presampling procedures on real-time PCR used for diagnosis of intramammary infections with Staphylococcus aureus in dairy cows at routine milk recordings. *Journal of dairy science,* **96(4):**2226-2233.

Mekibib, B., Furgasa, M., Abunna, F., Megersa, B. and Regassa, A., 2010. Bovine mastitis: prevalence, risk factors and major pathogens in dairy farms of Holeta town, central Ethiopia. *Veterinary World*, **3(9):**397-403.

Mekonnin, E.E., Awekew, A. and Naod Thomas, E., 2016. A Study on the Prevalence of Bovine Mastitis and Associated Risk Factors in and the Surrounding areas of Sodo Town, Wolaita Zone, Ethiopia. *Global Journal of Science Frontier Research*, **16(2).**

Metaferia, F., Cherenet, T.G., Abnet, F., Tesfay, A., Abdi, J. and Gulilat, W., 2011. A review to improve estimation of livestock contribution to the national GDP.

Moges, N., Asfaw, Y. and Belihu, K., 2011. A Cross Sectional Study on the Prevalence of Sub Clinical Mastitis and Associated Risk Factors in and Aronund Gondar, Northern Ethiopia. *International Journal of Animal and Veterinary Advances*, **3(6):**455-459.

Moon, J.S., Lee, A.R., Kang, H.M., Lee, E.S., Kim, M.N., Paik, Y.H., Park, Y.H., Joo, Y.S. and Koo, H.C., 2007. Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. *Journal of dairy science*, **90(3):**1176-1185.

NCCLS (National Committee for Clinical Laboratory Standards), 2000. Reference method for broth dilution antifungal susceptibility testing of conidial-forming filamentous fungi. Approved standard NCCLS M38-A.

Nickerson, S.C., 1994. Bovine Mammary Gland: structure and function; relationship to milk production and immunity to mastitis. Agri-Practice (USA).

NMC, 1990. Microbiological Procedures for the Diagnosis of Bovine Udder Infection. 3rd ed, Madison, Wisconsin: *National Mastitis Council (NMC), Inc:* 1-15.

NMC, 1999. Current Concept in bovine mastitis). 3rd.1840 Wilsonblud,Arlinton, VA 22201

NMC, 2002. Relationship of minerals and vitamins supplementation with mastitis and milk quality. Annual Meeting Proceedings, W. P. Weiss. National *MastitisCouncil (NMC), Inc*. **41:**37-44

NMC, 2011. Current status and future challenge in mastitis Annual Meeting Proceedings Hogeveen H., Pyorala S., Persson Waller K., Hogan J., Lam,Stephen T., Oliver P., Schukken Y., Barkema H., and Eric Hillerton J. *National Mastitis Council (NMC), Inc:*6-9.

Oliver, S.P. and Murinda, S.E., 2012. Antimicrobial resistance of mastitis pathogens. Veterinary Clinics of North America: *Food Animal Practice*, **28(2):**165-185.

Owens, W.E., Ray, C.H., Watts, J.L. and Yancey, R.J., 1997. Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. *Journal of dairy science*, **80(2):**313-317.

Paape, M.J. and Guidry, A., 1993. The teat defenses of mammary gland. *Advances in Experimental Medicine and Biology*, **225:**457-463.

Peeler, E.J., Otte, M.J. and Esslemont, R.J., 1994. Inter-relationships of periparturient diseases in dairy cows. *The Veterinary Record*, **134(6):**129-132.

Plozza, K., Lievaart, J.J., Potts, G. and Barkema, H.W., 2011. Subclinical mastitis and associated risk factors on dairy farms in New South Wales. *Australian veterinary journal*, **89(1-2):**41-46.

Poutrel, B., Boutonnier, A., Sutra, L. and Fournier, J.M., 1988. Prevalence of capsular polysaccharide types 5 and 8 among Staphylococcus aureus isolates from cow, goat, and ewe milk. *Journal of clinical microbiology*, **26(1):**38-40.

Quinn PJ, Carter ME, Markey BK and Carter GR., 2004. Clinical Veterinary Microbiology. Mosby publishing, London: **43-55**:327-344.

Quinn, P. J., M. E. Carter, B. Markey and Carter G. R., 1999. Clinical Veterinary Microbiology. Mosby Publishing, London. 327-344.

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R ., 1994 . Clinical veterinary microbiology, (Wolf publishing, London, England):327 – 344.

Radostits, O. M., C. C. Gay, K. W. Hinchcliff and Constable P. D., 2007. Veterinary Medicine: A Text book of disease of cattle, sheep, pigs, goats, and horses 10th edition, Ballier, Tindall, London:674-762.

Radostits, O. R., Blood, D. C., Bay, D. C., 1994. Veterinary Medicine: A Text Book of the Diseases of Cattle, Sheep, Goats, and Horses. 8thed. London: Bailler Tindall: 563- 614.

Radostits, O.M. Gay, G. C. Blood, D. C. and Hinchillif, K. W., 2000. Veterinary Medicine, 9th Edition, Harcourt Limited, London: 603-700.

Rahman, M.A., Bhuiyan, M.M.U., Kamal, M.M. and Shamsuddin, M., 2009. Prevalence and risk factors of mastitis in dairy cows. *Bangladesh Veterinarian*, **26(2):**54-60.

Rainard, P. and Poutrel, B., 1988. Effect of naturally occurring intramammary infections by minor pathogens on new infections by major pathogens in cattle. *American journal of veterinary research*, **49(3):**327-329.

Schalm, O.W., Carroll, E.J. and Jain, N.C., 1971. Bovine mastitis. In Bovine mastitis. A symposium.Philadelphia, USA: Lea & Febiger.

Shome, B.R., Das Mitra, S., Bhuvana, M., Krithiga, N., Velu, D., Shome, R., Isloor, S., Barbuddhe, S.B. and Rahman, H., 2011. Multiplex PCR assay for species identification of bovine mastitis pathogens. *Journal of applied microbiology*, **111(6):**1349-1356.

Snedecor, G.W. and Cochran, W.G., 1989. Statistical methods. Iowa State University Press: Ames, IA.

Sori, H., Zerihun, A. and Abdicho, S., 2005. Dairy cattle mastitis in and around Sebeta, Ethiopia. *Journal of Applied Research in Veterinary Medicine,* **3(4):**332.

Sylejmani, D., Ramadani, N., Robaj, A. and Hamidi, A., Prevalence and Antimicrobial Susceptibility of Bacterial Isolates from subclinical mastitis in the dairy farms in Kosovo. *Bulgarian Journal of Veterinary Medicine:* 1-9.

Tadesse, A. and Chanie, M., 2012. Study on the Occurrence of Bovine Mastitis in Addis Ababa Dairy Farms and Associated Risk Factors. *Advances in Biological Research,* **6(4):**151-158.

Tassew, A., Negash, M., Demeke, A., Feleke, A., Tesfaye, B. and Sisay, T. 2016. Isolation,dentification and drug resistance patterns of methicillin resistant Staphylococcus aureus from mastitic cow's milk from selected dairy farms in and around Kombolcha, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **8(1):**1-10.

Teklemariam, A.D., Ashebir, G., Tassew, A., Tesfaye, B. and Feleke, A., 2016. Isolation and phenotypic characterization of coagulase negative staphylococcus isolated from mastitic Cows in and around Zway town, Ethiopia. *International Journal of Fauna and Biological Studies,* **3(1):** 46-54

Tesfaye, A., Mekonnen, H., Rahwa, S. and Dendin, D., 2012. The Effect of Management Practices on Prevalence of Mastitis in Small Scale Dairy Farms in Nazareth, Ethiopia. *World Journal of Agricultural Sciences*, **8(2):**218-222.

Thrusfield, M., 2005. Veterinary epidemiology. 3rd edBlackwell Science. United Kingdom:158.

Tilahun, A.A., 2015. Prevalence of Bovine Mastitis in Lactating Cows and its Public Health Implications in Selected Commercial Dairy Farms of Addis Ababa. *Global Journal of Medical Research,* **15(2).**

Tolosa, T., Verbeke, J., Ayana, Z., Piepers, S., Supré, K. and De Vliegher, S., 2015. Pathogen group specific risk factors for clinical mastitis, intramammary infection and blind quarters at the herd, cow and quarter level in smallholder dairy farms in Jimma, *Ethiopia. Preventive veterinary medicine,* **120(3):**306-312.

Tsegaye, B., Tekuash, R. and Abdurhaman, Y., 2015. Prevalence and Potential Risk Factors of Bovine Mastitis in Selected Dairy Farms of Dire Dawa Town, Eastern Ethiopia. *Applied Journal of Hygiene,* **4 (1):** 06-11.

WHO, 2012. Antimicrobial resistance: frequently asked questions, Issued by the World Health Organization Communicable Diseases Cluster. Data retrieved from: ww.who.int/drugresistance/amrq&a.pdf

Workineh, S., Bayleyegn, M., Mekonnen, H. and Potgieter, L.N.D., 2002. Prevalence and aetiology of mastitis in cows from two major Ethiopian dairies. *Tropical Animal Health and Production,* **34(1):**19-25.

Yohannis, M. and Molla, W., 2013. Prevalence, risk factors and major bacterial causes of bovine mastitis in and around Wolaita Sodo, Southern Ethiopia. *African Journal of Microbiology Research,* **7(48):**5400-5405.

Yoseph, M., Azage, T., Alemu, Y. and Umunna, N.N., 2000. Reproductive management and reproductive performance of dairy herds in urban and peri-urban dairy production systems in Addis Ababa milk shed. *Pastoralism and Agro-pastoralism which way forward*: 312-319.

Zadoks, R.N. and Schukken, Y.H., 2006. Use of molecular epidemiology in veterinary practice. Veterinary Clinics of North America: *Food Animal Practice*, **22(1):**229-261.

Zenebe, N., Habtamu, T. and Endale, B., 2014. Study on bovine mastitis and associated risk factors in Adigrat, Northern Ethiopia. *African Journal of Microbiology Research*, **8(4):**327-331.

Zeryehun, T., Aya, T. and Bayecha, R., 2013. Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around Addis Ababa, Ethiopia. *J. Anim. Plant Sci*, **23(1):**50-55.

ANNEXES

Annex 1: Questionnaire format

Date ----------

Code ----------

Questioner format

1. Name of the owner ----------Sex-----Age---------Kebele----------
2. Education level of the owner of the farm----------
3. Marital status ----------
4. **Environmental factor**

4.1. Type of farming

A. Open(outdoor) B. Close(indoor)

4.2. Number of lactating cow per herd ----------

4.3. What the flooring system of the farm?

A. Soil D. Wood

B. Concert E. Other

C. Stone

4.4. What type of bedding is used for your milking cows?

A. Straw D. Sand

B. Sawdust E. No bedding

C. Shavings wood

4.5. Frequency of cleaning of cows environment per day

A. Once a day C. Three times a day

B. Twice a day D. Other

4.6. Frequency of body washing cow?

A. Every week D. >30 days

B. 15-30 days E. Every day

C. Not washed

4.7. Dose the farm use concentrates for cows feeding? A. Yes B. No

4.8. Do you follow milking order? A. Yes B. No

4.9. Dose the farm use pre milking udder preparation? A. Yes B. No

4.10. Dose the milker use drying towel? A. Yes B. No

4.11. Dose the milker use separate drying towel? A. Yes B. No

4.12. Dose the milker wash and disinfect his or her hand before and between milking of adjacent lactating cows? A. Yes B. No

4.13. Dose the milker use post milking teat disinfection or teat dipping?

A. Yes B. No

4.14. Frequency of milking cow?

A. Once a day

B. Twice a day

C. Three times a day

4.15. Do you have idea about subclinical mastitis? A. Yes B. No

4.16. Type of milking A. Striping B. Squeezing

4.17. Do you give feed after milking? A. Yes B. No

Annex 2: Procedure for cow side CMT test

1. A small amount of milk from each quarter was squired into shallow cups in the CMT paddle
2. Add equal amount of 3% CMT reagent to each cup and mixed well
3. Apply a gentle circular motion to a mixture in a horizontal plane for 15 s.
4. Interpreted the test result based on the thickness of the gel formed by CMT reagent and milk mixture and scored as negative (0), Trace (T), + (weak positive), ++ (distinctive positive), and +++ (strong positive).

Interpretation: The test results are interpreted subjectively as either 0 (negative), T (trace), +1, +2 or+3 based on the viscosity of the gel formed by mixing the reagent with the milk.

Score	Interpretation	Visible reaction
0	Negative	Milk fluid normal
T	Trace	Slight precipitation
+	Weak positive	Distinct precipitation but no gel formation
++	Distinct positive	Mixture of thickness with gel formation
+++	Strong positive	Viscosity greatly increased, strong gel i.e. cohesive with a convex surface

Annex 3: Procedure for milk sample collection

1. Prepare sterile universal bottle with tight fitting screw caps
2. Cleaned and dry udder and teats before sample collection
3. Wash the teats with soap and water and disinfect with 70% ethanol
4. Removed first few streams of milk and discard to reduce the number of contaminating bacteria in the teat canal.
5. To reduce contamination of the teat ends during sample collection, sample the near teats first, then the far ones
6. Remove the cap from the sample vial without touching its inner surface

7. Hold the vial horizontally and by turning the teat to a near horizontal position then the streams of milk direct in to the vial
8. Take precaution not to touch the teat end with the cap or the vial.
9. After sample collection; sample containing bottles, should be labeled and transported in an icebox to DBU microbiology laboratory.
10. Upon arrival, the samples should be stored in a refrigerator at 4°C until analyzed.

Annex 4: Procedure for Gram staining

1. Using a sterile inoculating loop, add 1 drop of sterile water to the slide and prepare a mixed smear
2. "Heat-fix" the slide with the specimen by passing it over a heat source
3. Place slide on the staining tray
4. Flood the fixed smear with crystal violet solution and allow for 1minute
5. Rinse off the crystal violet with distilled or tap water
6. Flood the slide with iodine solution and allow for one minute
7. Rinse off the iodine solution with distilled or tap water
8. Flood the slide with decolorizer (alcohol) for one to five seconds
9. Rinse off the decolorizer with distilled or tap water
10. Flood the slide with safranin (counter staining) and allow for 30 seconds
11. Rinse off the safranin with distilled or tap water
12. Dry the slide
13. Examine under microscope using a 100X objective lens.

Interpretation: Gram-positive bacteria stain deep violet to blue and gram-negative bacteria stain pink to red.

Annex 5: Procedures for Catalase test (slide test)

1. Place a small amount of growth from your culture onto a clean microscope slide.
2. Add a few drops of H_2O_2 onto the smear. If needed, mix with a toothpick.
3. DO NOT use a metal loop or needle with H2O2; it will give a false positive and degrade the metal.

Interpretation of the result: A positive result is the rapid evolution of O_2 as evidenced by bubbling. While a negative result is no bubbles or only a few scattered bubbles.

Annex 6: Procedures for Coagulase test (slide test)

1. Make a 1 inch diameter circles on a clean glass slide using a wax pencil
2. Place a drop of physiological saline in the circles on each end of a slide, or on two separate slides
3. With the loop, straight wire or wooden stick, emulsify a portion of the isolated colony in each drops to make two thick suspensions.
4. Add a drop of rabbit plasma to one of the suspensions, and mix gently
5. Look for clumping of the organisms within 10 seconds

Interpretation of the result: A positive result is the rapid clumping of organisms. While the negative result do not show any agglutination**.**

Annex 7: Procedures for CAMP TEST

1. Using an inoculating loop, streak a beta-lysin-producing *Staphylococcus aureus* in a straight line across the center of a sheep blood agar plate.
2. Streak test organism in a straight line perpendicular to the *S. aureus* with leaving 1cm space between the two streaks
3. Incubate the plate at 37 ^{o}C for 18-24 hours

Interpretation of the result: Positive: Enhanced hemolysis is indicated by an arrow head-shaped zone of beta-hemolysis at the junction of the two organisms. **Negative**: No enhancement of hemolysis.

Annex 8: Procedures for Indole test

1. Inoculate the bacteria in Tryptone broth and incubate for 24 hours at 37 ^{o}C
2. Add 15drops of Kovac's reagent in the incubated tube

Interpretation of the result: - Development of bright red color at the interface of the reagent after few seconds of adding the reagent is indole positive.

Annex 9: Procedures for antimicrobial sensitivity test

1. Transfer pure culture colonies in to sterile test tube of 5ml saline and make a suspension comparable with that of 0.5 % MacFarland standard.
2. Dip a sterile cotton swab in to the suspension and swap the whole surface of Mullar Hinton agar plate
3. Allow the surface the inoculated agar to dry for 5 minutes
4. Apply antimicrobial disc on the surface of inoculated agar plate using sterile forceps and press gently to ensure the complete contact with agar plate.
5. Incubate for 24 hours at 37 ^{0}C
6. Measure the inhibition zone using ruler in mm and interpret the result qualitatively as resistant, intermediate or susceptible based on standard protocol.

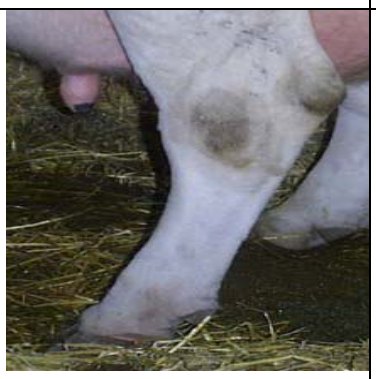
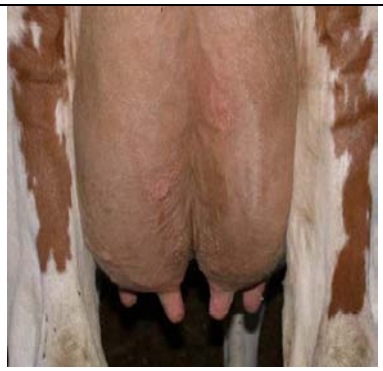
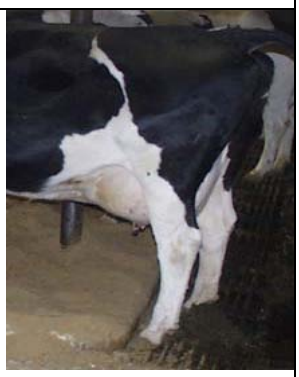
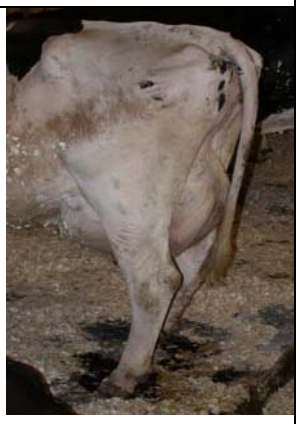
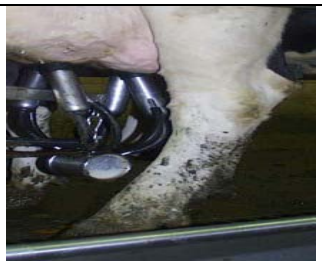
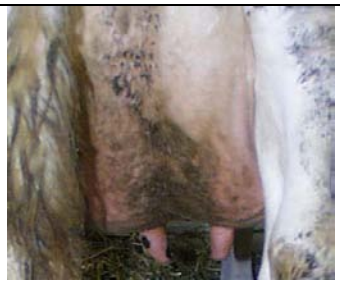
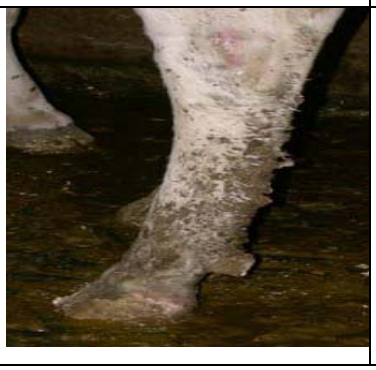
Annex 10: Zone of inhibitions interpretation chart

Table 12. Zone size interpretation chart (modified from NCCLS (1990) M2-A4)*

Disc content in µg unless otherwise stated	*Antimicrobial agents*	*Diameter of zone of inhibition to nearest mm.*			
		Resistant ≤	*Intermediate*	*Moderately Susceptible*	*Susceptible* ≥
30	AMIKACIN	14	15–16	–	17
20/10	AMOXICILLIN / CLAVULANIC ACID				
	when testing staphylococci	19	–	–	20
	when testing other bacteria	13	–	14–17	18
10	AMPICILLIN				
	when testing Gram-negative enteric bacteria	13	–	14–16	17
	when testing staphylococci	28	–	–	29
	when testing enterococci	16	–	≥17	–
	when testing non-enteric streptococci	21	–	22–29	30
100	CARBENICILLIN				
	when testing *Pseudomonas* spp.	13	–	14–16	17
	when testing other Gram-negative bacteria	19	–	20–22	23
75	CEFOPERAZONE	15	–	16–20	21
30	CEFOTAXIME	14	–	15–22	23
30	CEPHALOTHIN	14	–	15–17	18
30	CHLORAMPHENICOL	12	13–17	–	18
5	CIPROFLOXACIN	15	–	16–20	21
2	CLINDAMYCIN	14	15–20	–	21
10	ENOXACIN	14	–	15–17	18
15	ERYTHROMYCIN	13	14–22	–	23
10	GENTAMICIN	12	13–14	–	15
30	KANAMYCIN	13	14–17	–	18
30	MOXALACTAM	14	–	15–22	23
1	NAFCILLIN when testing staphylococci	10	11–12	–	13
30	NALIDIXIC ACID	13	14–18	–	19
30	NETILMICIN	12	13–14	–	15
300	NITROFURANTOIN	14	15–16	–	17
10	NORFLOXACIN	12	13–16	–	17
1	OXACILLIN when testing staphylococci	10	11–12	–	13
10 units	PENICILLIN G				
	when testing staphylococci	28	–	–	29
	when testing enterococci	14	–	≥15	–
	when testing non-enteric streptococci	19	–	20–27	28
100	PIPERACILLIN				
	when testing *Pseudomonas* spp.	17	–	–	18
	when testing other Gram-negatives	17	–	18–20	21
5	RIFAMPIN	16	17–19	–	20
10	STREPTOMYCIN	11	12–14	–	15
250/300	SULPHONAMIDES	12	–	13–16	17
30	TETRACYCLINE	14	15–18	–	19
75	TICARCILLIN				
	when testing *Pseudomonas* spp.	14	–	–	15
	when testing other Gram-negatives	14	–	15–19	20
10	TOBRAMYCIN	12	13–14	–	15
1.25/23.75	TRIMETHOPRIM-SULPHAMETHOXAZOLE	10	–	11–15	16
30	VANCOMYCIN				
	when testing enterococci	14	15–16	≥17	–
	when testing other Gram-positives	9	10–11	–	12

* Updated according to the Third Informational Supplement, December 1991.

Annex 11: Hygiene score card chart

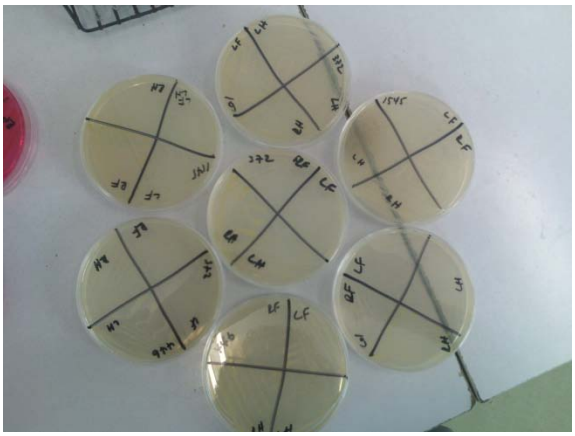
Score	Legs	Udder	Flank and upper leg
Score 1 Good			
Score 2 Good			
Score 3 Poor			
Score 4 Poor			

Source: http://www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf hygiene scoring card score sheet

Annex 12: Figures of isolated bacteria colonies and hemolysis on blood agar

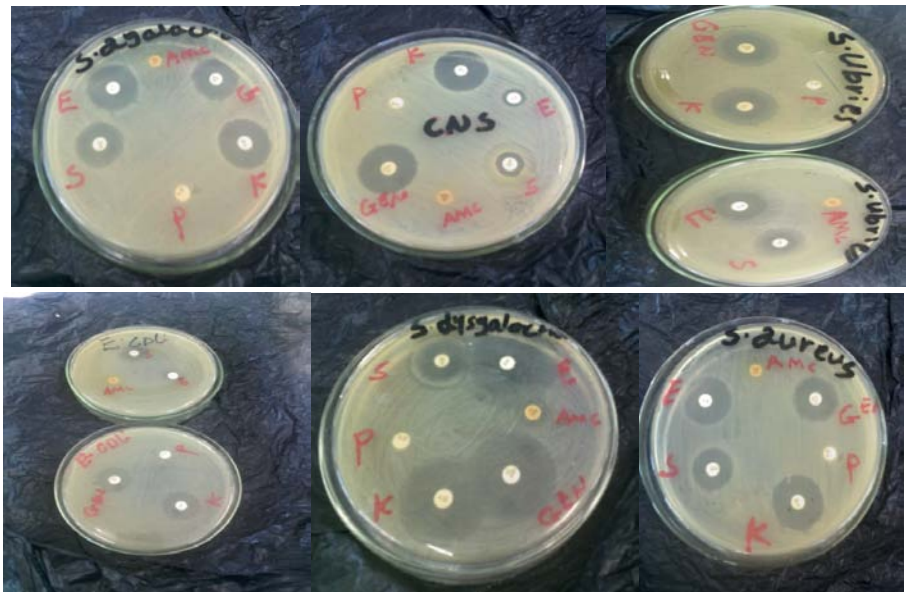
Annex 13: Figures of isolated *Staphylococcus* colonies on Mannitol salt agar

Annex 14: Figure of bacteria colonies on nutrient agar



Annex 15: Figure of *E.coli* colonies on MacConkey agar

Annex 16: Figures of Kirby Bauer disk diffusion antimicrobial sensitivity test



Annex 17: Figure of Catalase test

Annex 18: Figure of Coagulase test