Contagious Mastitis: Prevalence, Risk Factors and Antibiotics Susceptibility Profile Study in Jimma Dairy Farms, South- West Ethiopia

MSc Thesis

By

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Contagious mastitis: Prevalence, risk factors and antibiotic susceptibility profile study in Jimma Dairy farms, south -West of Ethiopia

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ABBREVIATIONS

CAMP test	Christie, Atkins and Munch-Peterson
CM	Clinical Mastitis
CMT	California Mastitis Test
CNS	Coagulase Negative *S.aureus*
CPS	Coagulase Positive *S.aureus*
CSA	Central Statistical Agency
DCC	Direct Cell Counter
IMI	Intramammary infection
JTSSMIDAO	Jimma town Small Scale Micro-industry & development administration
NCCLS	National Committee for Clinical Laboratory Standards
NMC	National Mastitis Council,
OPEDJZ	Office of Planning and Economic Development for Jimma Zone
S. agalactiae	*Staphylococcus agalactiae*
S. aureus	*Staphylococcus aureus*
S. dysgalactiae	*Streptococcus dysgalactiae*
S. intermedius	*Staphylococcus intermedius*
S. uberis	*Streptococcus uberis*
S.chromogenes	*Staphylococcus chromogenes*
S.*Hyicus*	*Staphylococcus hyicus*
SCC	Somatic Cell Count
SCM	Subclinical Mastitis
SEs	Staphylococcal Enterotoxins
Spp.	Species
SPSS	Statistical Package for the Social Sciences.
SVARM	Swedish veterinary antimicrobial resistance monitoring
T	Trace
TSST	Toxic shock syndrome toxin.
USA	United States of America.
WBC	White blood cells

ABSTRACT

A study was conducted in Jimma town cross breed dairy farms to determine the prevalence contagious mastitis, risk factors and antimicrobial susceptibility profile of the isolates between October 2012 and May 2013 using California Mastitis Test (CMT) for screening subclinical mastitis. All milk samples were cultured for bacteriological identification by following the protocol described by National Mastitis Council. Clinical mastitis prevalence was determined through examination of abnormalities of milk, udder or cow during sample collection. Out of the 206 cows examined 101 cows were positive to mastitis. Out of 824 quarters examined, 50 (6.06%) were found blind and 774 quarters were found to be functional. Out of the total quarters examined, 404 (52.2%) were affected, 34 (8.4%) clinically and 370 (91.6%) sub clinically. Of 404 infected quarters, 57 quarters were found positive to contagious mastitis. The overall prevalence of contagious mastitis at cow and quarter level was 27.7% and 7.4% respectively. Potential risk factors for the occurrence of contagious mastitis were wood or soil floor type, source of water, milkers, lactation stage and purchasing heifers into herd. The pathogens isolated in this study were *S. aureus and S. agalactiae,* S. *aureus* was the most dominant species identified in this study area. Antimicrobial susceptibility tested was conducted on 57 isolates against seven antimicrobial agents for *S.aurues* and nine for *s.agalactae.* All strains were resistant to Amoxacilline +CLAV(30+15µg), Cefquinome, Streptomycin, Tetracycline (80µg), Trimethoprim +Sulfa(5.240µg) and polymyxin by 82.5% 3.5% ,7.0% ,42.1%, 3.5%,82.5% respectively. About 18.8% of *S.agalactae* isolates was resistant to Ampicillin (30 µg) and Enrofloxacin (10 µg) were resistant to. Good hygiene in milking process, creation of awareness for milkers on contagious mastitis, milking clinically infected cows at last, culling chronic mastitis carriers, treating clinically infected cows and dry period therapy could reduce the prevalence of contagious mastitis in the study area.

Keywords: antibiotic susceptibility, contagious mastitis, Cross breed, Dairy farm, Jimma town, Prevalence, *Staph ylococcus aureus, Streptococcus agalactae.*

1. INTRODUCTION

Mastitis is an inflammation of the udder resulting from the invasion of pathogenic microorganisms, and it is the most costly disease in dairy cattle industry (Greer and Baker, 1992). It reduces milk and milk products in all dairy producing countries of the world (International Dairy Federation, 1999). In addition to heavy losses in milk quality and quantity, it also causes irreversible damage to the udder tissue and less occasional fatalities (Radostits *et al*., 2000). In general, mastitis is a complex disease dealing with, the interaction of microorganisms and the cow's anatomy and physiology, dairy husbandry and management, milking equipment and procedures and environment (Woods, 1986).

Mastitis is classified into two forms, based on symptoms: Clinical mastitis (CM) and Subclinical mastitis (SCM). It can also be divided either into an acute or a chronic form, based on the time course of disease. The former categorization is important in order to decide the right way of treatment and prevention (Sandholm, 1995). Clinical mastitis is characterized by *visible symptoms* (general: fever and debilitation; local: udder redness, swelling, heat and pain, and milk cloth or other macroscopic milk transformations). These symptoms are graded according to severity (mild, moderate or severe). Clinical mastitis is, therefore, because of the visible symptoms, often uncomplicated to diagnose. On the contrary, detection of SCM is a more demanding process, SCM is mastitis *without clinical/visible symptoms*. To diagnose SCM, one, therefore, has to use laboratory methods (Sandholm, 1995). Though mastitis is commonly manifested as subclinical, clinical mastitis is also commonly observed, which could be manifested as per acute, acute or chronic forms (Radostits *et al*., 1994).

In the case of SCM there is no visible sign of the disease, but somatic cell count will be elevated and bacteria will be detected in the milk sample culture. Subclinical mastitis

causes the greatest financial loss to dairy farms through lowered milk production (Crist *et al*., 1997).

The cow udder is an ideal environment for microbial growth and under optimum udder conditions, such as temperature, nutrition, and freedom from outside influence, pathogenic organisms multiply astronomically and it is this factor that causes udder damage and triggers the response that is recognized as mastitis (Woods, 1986). Broadly, cow -to-cow or contagious, environmental, infection in dry cows (example, summer mastitis) and to a lesser extent uncommon causal types of mastitis infection can be recognized (Radostits *et al*., 1994).

Staphylococcus aureus present in the udders of chronically infected cows and also in cuts and chaps on the teat skin, *Streptococcus agalactiae* found only in the udder, though it can survive for 2-3 weeks away from the cow without multiplication and *Streptococcus dysgalactiae* found in the udder and on teat skins are the main pathogenic bacteria that are involved in contagious mastitis (Blowey, 1990; Radostits *et al*., 1994; Radostits *et al.,* 2000).

In Ethiopia, livestock represents a major national resource and form an integral part of agricultural production system. Cows represent the largest population of cattle production of the country; 42% of the total cattle are milking cows (Gebrewold *et al*., 2000). Per capital consumption of milk in Ethiopia is as low as 17 kg per head while the average figure for Africa is 26 kg per head (Gebrewold *et al*., 2000). The quality and quantity of milk production deteriorate due to biological causes including the low genetic potential of the animals, poor nutrition and prevalence of diseases (Atyabi *et al.,* 2006). Mastitis is among the factors contributing to reduced milk production (Biffa *et al.,* 2005), and it is among the most important diseases in dairy animals with worldwide distribution (Zhao and Lacasse, 2007). Mastitis has been known to cause a great deal of loss or reduction of productivity. It influences the quality and quantity of milk, and causes culling of animals

at an unacceptable age (Mungube *et al*., 2005; Gebreyohannes *et al,* 2010). In Ethiopia, the prevalence and risk factors of bovine mastitis have been reported from different parts of the country in different times (Alma *et al.*, 2008; Getahun *et al.*, 2008, Lakew *et al.,* 2009; Abera *et al.,* 2012). However, there no published data on the prevalence, risk factors and antimicrobial susceptibility test of contagious mastitis in bovine particularly in Jimma area. Hence, the objectives of the study were to assess the overall prevalence of contagious mastitis in crossbreed dairy farms, to isolate and identify the causative pathogens of contagious mastitis, to assess risk factors of contagious mastitis and to assess the antibiotic susceptibility pattern of the isolates in Jimma dairy farms, Oromiya Regional State, South-West Ethiopia.

2. LITERATURE REVIEW

2.1 Contagious Mastitis

2.2. Mastitis

When studying the literature on mastitis, difficulties are constantly encountered because the concepts "normal", "udder infection", "subclinical" and acute mastitis are insufficiently delineated. According to Schalm *et al*. (1971) the term "mastitis" is derived from the Greek word mastos meaning breast and the suffix "itis" meaning inflammation of. Thus, mastitis means inflammation within the mammary gland. Detailed and comprehensive definition of mastitis is given by Faull and Hughes (1985) as:

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

Subclinical mastitis a quarter with pathogens and many neutrophils in the milk, but the milk looks normal and the quarter feels normal.

Clinical mastitis:

Acute mastitis is when there are obvious signs of inflammation of the udder such as heat, pain and swelling. The milk is macroscopically abnormal and the animal may have feverish temperature.

Subacute mastitis is when there are no obvious changes in the udder but when there are persistent clots especially in the foremilk.

Mastitis is an inflammation of the mammary gland caused by microorganisms, usually bacteria that invade the udder, multiply and produce toxins that are harmful to the gland (Crist *et al*., 1997).

Contagious mastitis is an intramammary infection (IMI) transmitted directly from cow to cow by pathogens for which the udder is the primary reservoir. It tends to be subclinical in nature. The economic impact of this form of mastitis is mostly due to production loss,

reduced milk quality (high SCC), premature culling and the eventual cost of control programs (Erskine, 2001).

2.3 Etiologies of Contagious Mastitis

Mastitis is a disease of many mammalian species. At least 137 infectious causes of bovine mastitis are known to date and in large animals the commonest pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, other *streptococcus* and Coliforms (Fraster, 1986).

Causal agents of mastitis could also be classified in to two based on sources of infections, namely, 1. Contagious caused by *Streptococcus agalactiae* and *Staphylococcus aureus* 2. Environmental mastitis caused by Coliforms- *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca* and *Enterobacter aerogenes* and Environmental *Streptococci- Streptococcus uberis,* and *Streptococcus bovis* and *Enterococcus faecium* and *Enterococcus faecalis* (Crist *et al*., 1997).

The most common pathogens of contagious mastitis are *Staphylococcus aureus, Streptococcus agalactiae*, and *Mycoplasma* species that reside primarily in the cow's udder. Among the contagious pathogens, the most common are *S. aureus* and *S. agalactiae*. The major reservoir for these pathogens is the infected udder, and infections are spread among cows or between quarters during the milking process by contaminated milking equipment, milker's hands, or cloths or sponges used to wash or dry more than one cow. Infections tend to be chronic and subclinical with periodic clinical episodes. Thus contagious mastitis results in decreases in milk production and increases in bulk tank SCC, but there may be few visible symptoms, i.e. this tends to be a hidden form of mastitis. Herds with high bulk tank SCC tend to have high levels of infections by contagious pathogens (Allore, 1993).

2.3.1. *Staphylococcus aureus*

They are gram-positive cocci which tend to form clusters and pairs, catalase positive and are ß-hemolytic (Quinn *et al.,* 1994). It can cause subclinical and clinical mastitis in dairy cows, and is usually associated with elevated SCC (Wilson *et al.,* 1997) and damage to the secretory mammary epithelial cells. New infections are controlled by adopting measures like proper milking procedures, improved milking hygiene and housing management (Arnold, 2011).

Staphylococcus aureus is found in the udders of chronically infected cows and also in cuts and chaps on the teat skin (Blowey, 1990). About 10% of the cows may have clinical mastitis but another 50% can have subclinical mastitis and act as a source of infection for further clinical cases (Quinn *et al*., 1999). It is not an obligate inhabitant of the mammary gland and is the worst of the contagious bacterial organisms causing chronic deep infection of the mammary gland causing fibrosis and abscess, which is extremely difficult to cure. It is very difficult to cure the infections once it is established and chronic infections are resistance to antibiotics (Rebhun, 1995). Many cases are characterized by slowly developing indurations, atrophy with the occasional appearance of clots in the milk or wateriness of the first streams (Radostits *et al.*, 1994). The type of mastitis ranges from subclinical to the peracute life threatening form, one of which is gangrenous mastitis caused by the action of Alpha toxin that damages the blood vessels resulting in ischemic coagulative necrosis of the adjacent tissue (Quinn *et al*., 1999).

Staphylococcus aureus, typically more pathogenic than *S. agalactiae*, causes a greater reduction in milk yield, clinical signs of mastitis, and a variable SCC. Intramammary infections commonly result in micro abscesses in the mammary gland, which make antibiotic therapy less successful. Chronic infections with *S. aureus* are common and likely to recur in subsequent lactations (Roberson, 1999).

2.3.2 *Streptococal* angents

Streptococcus agalactiae is a gram-positive bacterium, cocci which tend to form chains, it is a contagious obligate parasite of the bovine mammary gland, continues to be a major cause of sub-clinical mastitis in dairy cattle. It is associated with elevated SCC and total bacteria count and a decrease in the quantity and quality of milk products produced (Keefe, 1997).

They are capable of adhesion to epithelial cells, and do not actively invade gland tissue, but multiply in the milk and on the mucosa of the teat cistern and gland ducts. They do not produce toxins on a comparable scale to *S. aureus*, but do secrete an irritant which induces an inflammatory reaction. They are pyogenic and ß-hemolytic (Schalm *et al.,* 1971; Fox and Gay, 1993)**.**

Most *S. agalactiae* infections can be treated effectively with appropriate intramammary antibiotics, but some chronic cases may not resolve (Lombard *et al.,* 2008). *Streptococcus agalactiae* is the classic example of contagious mastitis, because it is highly contagious and an obligate inhabitant of the mammary gland. The agent can survive for 2-3 weeks away from the cow but multiplication occurs only in the udder (Blowey, 1990). The bacterium does not invade the glandular tissue (Quinn *et al*., 1999) and hence doesn't cause fibrosis and abscess. Streptococcal mastitis is largely subclinical with occasional acute flare-ups. It will permanently decrease productivity in the affected gland in chronic infections (Rebhun, 1995).

Streptococcus dysgalactiae is found in the udder and teat lesions (Blowey, 1990) and tends to have a lower prevalence than *Streptococcus agalactiae* and may become overtly clinical *(*Rebhun, 1995). It is generally characterized as an environmental pathogen, but also may have characteristics of a contagious organism and appears to spread from cow to cow. This pathogen is generally responsive to teat dipping and dry cow therapy, but

new infections can occur in a herd when no other udder infections by this organism are present (Smith and Hogan, 1995).

2.3.3 *Mycoplasma* species

Cows of all ages and all stages of lactation can be affected by mycoplasmal mastitis; however, those that have recently calved show the most severe signs. These can be due to the long-term persistence of the organisms in the udders (up to 13 months) and some cows may become shedders of mycoplasma without severe clinical signs (Qiunn *et al*., 1999).

Mycoplasma bovis is the most common cause and *Mycoplasma californicum* and other species have been isolated from the milk. *Mycoplasmal* species cause herd endemics of acute mastitis that subsequently evolve into chronic mastitis. Following acute attack cows may show chronic mastitis, intermittent acute flare-ups or have subclinical infection requiring culture confirmation (Rebhun, 1995).

2.4 Risk Factors of Contagious Mastitis

Risk factors such as management practices (poor teat and udder hygiene, poor environmental hygiene, sanitation, large herd size, use of hand washcloth) and diet (selenium and vitamin E deficiency) amongst others have been reported to be important in the prevalence and epidemiology of subclinical mastitis (Bartlett *et al.,* 1992; Chassagne *et al.,* 1998). Teat dipping and dry cow therapy have also been found to play key roles in preventing subclinical and clinical mastitis (Hogan *et al.,* 1994; Lam *et al.,* 1995).

Herds that have a large number of cows infected with contagious mastitis pathogens are likely to have more heifers infected at their first calving. Prevalence of mastitis can also be affected by housing decisions; in group housed calves opportunity for cross suckling

may increase risk for transmission of contagious pathogens (McDougall et al., 2009). Herd-Level risk of infection may also depend on herd management factors (Barkema *et al.,* 1998; Peeler *et al.,* 2000) and on exposure to infected herd mates (Lam *et al.,* 1997; Zadoks *et al.,* 2001).

Cow characteristics that influence the susceptibility to mastitis include parity, stage of lactation, and genetic make-up (Barkema *et al.,* 1998; Busato *et al.,* 2000; Schukken *et al.*, 1999). This implies differences between cows in susceptibility to mastitis, or within cow-transmission of causative agents (Barkema *et al.,* 1998).

Quarter-level factors that affect susceptibility to mastitis include SCC and infections with minor pathogens (Hogan *et al*., 1988; Lam *et al.,* 1997;Schukken *et al.,* 1999). Quarter position has been described as a risk factor in studies on incidence of clinical mastitis and prevalence of subclinical mastitis (Barkema et al., 1998; Busato *et al.,* 2000). The IMI was found more often in rear quarters than in front quarters. Teat dipping and dry cow therapy have also been found to play key roles in preventing subclinical and clinical mastitis (Hogan *et al.,* 1994; Lam *et al.,* 1995).

The sampling unit in risk factor studies can be herd, cow, or udder quarter and the outcome of interest can be SCC, clinical mastitis (Peeler *et al.,* 2000), subclinical mastitis (Busato *et al.,* 2000), or IMI, i.e., the combination of clinical and subclinical mastitis (Lam *et al.,* 1997).

2.5 Transmission of Contagious Mastitis

Contagious mastitis can be transmitted from one cow to another by pathogens for which the udder is the primary reservoir during milking process and new infections are most often acquired during the lactation period. Incidence of mastitis caused by contagious pathogens depends on the dose, type of microbes to which a cow is exposed, physical

barriers and the innate and acquired immunity. Contagious microorganisms are well adapted to survival in the udder and usually establish mild clinical infections of long duration (chronic infections) (Erskine, 2001).

Staphylococcus aureus is one of the most prevalent contagious mastitis pathogen that colonizes the teats during damage to the skin surface. Transmission of *S. aureus* infections occur mainly through contaminated milking machines, udder wash equipment, and the hands of milking machine operators. It can survive outside the cow for a shorter period of time. Infections caused by *Staphylococcus aureus* are mostly sub-clinical with periodic flare-up of clinical symptoms. Chronic infection of heifers can serve as a source of new infection in the herd. The frequency of the *S. aureus* infections is related to age of the cow. Culling, grouping and dry cow therapy helps to fight *Staphylococcus aureus* infections in a herd (Svensk mjolk, 2003).

Streptococcus agalactiae can spread throughout a herd from a single infected animal. The infected udder is the most important reservoir for this bacterium. They are transmitted to uninfected quarter mainly at milking time. Contaminated milking machines, udder wash cloths, and the hands of machine operator also transmit these bacteria. Breakdowns of contagious mastitis are usually due to the introduction of infected animals to the herd, or the employment of men who carry infection with them (Radostits *et al.,* 2000). The infections are mainly sub-clinical (National Mastitis Council, 1996) and there are most frequent in the younger age groups (Radostits *et al.,* 2000).

2.6 Response of the Udder to Infection

Infections of the mammary gland by pathogenic bacteria result in decreases in milk production and compositional changes that vary with the intensity and duration of the infection (Eberhart *et al.,* 1987; Harmon, 1994). Compositional changes include decreases in lactose, fat, casein, and calcium and increases in sodium, chloride, and blood

proteins in milk. Subclinical infections are those with no visible changes in the appearance of the milk or the udder, but milk production decreases, bacteria are present in the milk, and composition is altered. Clinical mastitis is characterized by abnormal milk and swelling or pain of the udder; it may be accompanied by systemic signs such as elevated rectal temperature, depression, or decreased feed intake. As in subclinical mastitis, milk production declines, bacteria are present in the milk, and dramatic changes in milk composition are usually present. Chronic mastitis is an infection that is long duration and may show periodic clinical symptoms (Kitchen, 1981).

One of the early events of an infection is the movement of white blood cells or leukocytes into the udder to fight the infection (Harmon, 1994). The end result is an increase in the number of cells or somatic cell count (SCC) in milk. All milk contains cells, but the number of cells depends on the presence or absence of infection. The normal SCC of milk from uninfected cows is less than 200,000 per ml. Milk from first lactation cows may be below 100,000 per ml. Thus the SCC of milk from individual quarters, individual cows, or from the bulk tank is commonly used as an indicator of udder health (Eberhart *et al.,* 1982). A simple cow side screening test to indicate relative somatic cell level for individual cows is the California Mastitis Test (CMT), sometimes called a paddle test (Eberhart *et al*., 1987).

In case of mastitis counts of *Streptococci*, *Staphylococci* or coliforms will be as high as the total plate count and can be very high up to 10^7 cfu/ml. Bulk milk count may even increase to 10^5 cfu/ml under certain circumstances (Slaghuis, 1996).

2.7. Diagnosis

2.7.1 Somatic cell count (SCC)

One can always find some somatic cells, even in milk from a healthy udder (Saloniemi, 1995). In a healthy udder, the milk cells mainly consist of macrophages, lymphocytes and epithelial cells. Epithelial cells in milk are eliminated cells from the inner parts of the

udder and are part of the natural, ongoing renewal of the body cells. When the udder tissue is exposed to an infection, the levels of neutrophilic granulocytes will increase as an effect of the rapid recruitment of inflammatory cells to the site of inflammation (Andersson *et al*., 2011; Sandholm M., 1995). This raise of the somatic cells in the milk can be counted, using different tests, and the result can be used as an indicator for udder health at cow level and prevalence of SCM at herd level (Andersson *et al*., 2011). The most important factor increasing the SCC in the milk of a single cow is an infection caused by bacteria (Andersson *et al*., 2011). However, other factors can also affect the SCC directly: noninfectious mastitis; and time of the day. There are also factors that make the cow more sensitive to infection and therefore indirectly affect the SCC: lactation stages; age/parity of the cow; breed; temperature and season; stress; and care factors. This must always be taken into consideration, as well as the daily milk yield since it also affects the SCC – cows with a low milk production can, due to a concentration effect, naturally have an increased SCC. Generally, a healthy udder is considered to have less than 100 000 cells/ml and a healthy quarter less than 50 000 cells/ml, which is somewhat less than the cut-off level for a negative CMT ("CMT 1", corresponding to ≤200 000 cells/ml) (Andersson *et al*., 2011; Brolund, 1985; Forsback *et al.*, 2009; Saloniemi, 1995). At herd level, EU regulations stipulate that the SCC should not exceed 400 000 cells/ml (as an average value over a three month period, with at least one milk sample per month). Considering acceptable bulk tank SCC levels, the cut-off limit somewhat differs between different regulations. In New Zeeland and the EU, the regulations stipulate less than 400 000 cells/ml, while in Canada the limit is less than 500 000 cells/ml and in USA less than 750 000 cells/ml.

The contagious mastitis pathogens *Staphylococcus aureus, Streptococcus agalactiae*, and *Mycoplasma spp*. reside primarily in the cow's udder; therefore, when they are found in bulk milk, these mastitis causing organisms are strong indicators of the presence of intramammary infections in the herd (Gonzalez *et al.,* 1986; Fox *et al*., 2005; Rodrigues *et al.*, 2005).

2.7.2 California mastitis test (CMT)

The California Mastitis Test (CMT) is used on farms to identify subclinical mastitis by an indirect estimation of the SCC in milk (Leach *et al.,* 2008). A bromocresol-purple-containing detergent is used to break down the cell membrane of somatic cells and the subsequent release and aggregation of nucleic acid forms a gel-like matrix with a viscosity that is proportional to the leukocyte number. It is cost effective, rapid, user friendly and can be used 'on-site' or in the laboratory. It can be difficult to interpret and has low sensitivity (Viguier *et al.,* 2009).

The 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 a negative, trace, 1+, 2+ or 3+ inflammatory response based on the viscosity of the gel formed by mixing the reagent with milk (Radostits *et al.,* 1994).

Fresh unrefrigerated milk can be tested using the CMT for up to 12 hours. Reliable readings can be obtained from refrigerated milk for up to 36 hours. If stored milk is used, the milk must be thoroughly mixed prior to testing because somatic cells tend to segregate with milk fat. The CMT reaction must be scored within 15 seconds of mixing because weak reactions will disappear after that time. The degree of reaction between the detergent and the DNA of nuclei is a measure of the numbers of somatic cells in milk. The threshold for CMT scores depend on the objective of the study. If it is used to minimize the rate of false negatives, the test should be read as negative versus positive with trace scores regarded as/ recorded as positive. If the CMT is to be used in culling

decisions, a threshold with a lower rate of false positives may be desirable (Larsen, 2000).

2.7.3 Culture

The microbiological examination of both individual cow and bulk tank culture are elements of mastitis control. Most mastitis control programs include the use of individual cow cultures to determine which mastitis pathogens are present on the farm. 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).

There have been a number of studies to improve culture quality in identification of intramamary infection. Comparisons were made on pre and post milking samples, pre culture incubation, pre culture freezing, increased plate inoculation volumes, frequency of sampling and centrifugation (Dinsmore *et al*., 1992).

Sears *et al.*(1991) using both pre-milking and post-milking positive results as definitive diagnosis ("gold standard"), found sensitivities of 92, 86 and 99% for S*taphylococcus aureus,* coagulase negative Staphylococci and for Streptococcus species other than S*treptococcus agalactiae* in pre-milking milk samples, respectively. Similarly, for post-milking samples the corresponding values were 96, 98, and 99%. The sensitivity was higher in pre-milking samples although multiple isolates were more common.

2.8 Control and Prevention of Contagious Mastitis

Monitoring udder health is an important component of mastitis control (Radostitis *et al.*, 1994). A regular assessment of udder health status is available through the use of somatic cell count (SCC) data. By setting goals for udder health status, it is easy to measure the success of udder health management programs or interventions. The practical use of SCC data to determine cow infection status requires the selection of a threshold level, which used to classify infected and healthy quarters or cows (Dohoo and Meek, 1982). Emanuelson (1997) used a threshold of 200,000 cell/ml at cow level in monitoring udder health status in Sweden. According to Dohoo and Meek (1982) 300,000 cells/ml and 250,000 cells/ml can be used to identify infected quarters and cows, respectively.

Prevention is the key to controlling mastitis, not treatment. Treatment is an attempt to eliminate an infection that has already occurred and should be limited to clinical cases. Mastitis control based solely on antibiotic therapy during lactation is both costly and ineffective. Prevention is based on reducing the number of bacteria to which the teat end is exposed; for contagious pathogens this involves reducing cow to cow spread. Effective control measures differ for contagious and environmental pathogens (Eberhart *et al.*, 1987; Hogan and Smith, 1987).

2.8.1 Teat Dipping and Dry Cow Therapy

Teat dipping and dry cow therapy form the basis of mastitis prevention programs (Smith and Hogan, 1995). An effective germicidal teat dip should be applied to all teats at the end of milking to kill contagious bacteria that were deposited on the skin during milking. This is the single most effective method of preventing new infections by contagious pathogens. Teat dipping alone can reduce new infections by contagious bacteria by 50%. Examples of some teat dips that have historically been effective are those that contain 0.5 to 1.0% iodine (iodophor), 4.0% hypochlorite, or 0.5% chlorhexidine acetate.

Dry Cow Treatment protects udders from new infections in the dry period, directly through the effect of the antibiotic and indirectly by promoting the formation of a natural keratin plug that seals the teat canal (Williamson et al 1995).

2.8.2 Milking Time Hygiene

Strict milking time hygiene is a high priority to reduce spread of contagious bacteria from one cow to another and to reduce bacterial contamination of the bulk tank milk. Many acceptable practices may be used to prepare the teats for milking. However, teats should be clean and dry before applying the milking unit. The use of a germicidal udder wash is recommended, but rinsing a contaminated cloth or sponge in a germicide solution will not kill all the bacteria in the cloth. Anything that causes liner slippage may increase the chance of spread of infections from one infected quarter to other quarters of the same cow (Sordillo, 1995).

2.8.3 Pre- dipping

Pre dipping, i.e. use of germicidal teat dip before milking to sanitize the teat, has been shown to reduce environmental mastitis by 50% (Pankey *et al.,* 1987). However, pre dipping has not reported benefit in controlling contagious pathogens, because contamination of teats will occur after its use. Teat dipping with germicidal or barrier teat dips during the dry period has no added benefit in preventing mastitis (Matthews *et al.,* 1988; Schultze, 1985; Galton *et al.,*1986).

2.8.4 Culling

Is a necessary part of control of *mycoplasma* mastitis and will play an important role in reducing the number of *S. aureus* infected cows in a herd (Fox and Gay, 1993; Smith and

Hogan, 1995). Culling chronic mastitis cows which have not responded to treatment eliminates a source of contagious bacteria (the infected cow), reducing the risk of new infections in other cows. The level of infection in the herd and the bulk tank SCC are immediately affected. However, culling decisions should be made after effective milking time hygiene and teat dipping programs are in place in order to gain long term benefits.

2.8.5 Segregation

Herds with a significant *S. aureus* or *mycoplasma* problem may benefit from a program of segregating infected cows and milking them last (Fox and Gay, 1993). The success of segregation appears to be dependent upon the adequate identification of infected cows; this is usually done by culturing of milk samples and can be quite expensive (Stamp, 1994).

2.8.6 Lactational Therapy of Clinical Mastitis

Antibiotic therapy during lactation has generally been successful in *S. agalactiae* problem herds. In contrast, the treatment of *S. aureus* infected cows during lactation is of limited value, because cure rates are usually less than 50%. Antibiotic therapy of clinical cases may reduce clinical symptoms but does little to improve the prevalence of infections in the herd; most of the udder infections are subclinical and go undetected. *Mycoplasma* infections are not responsive to antibiotic therapy (Eberhart *et al.,* 1987; Fox and Gay, 1993; Smith and Hogan, 1995; Smith *et al.,*1998).

2.9 Treatment of Contagious Mastitis

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).

Since mastitis results in the destruction and disturbances of the mammary gland and affects milk production and productivity, it needs serious and immediate action as soon as possible. Among the many actions that could be taken as treatment, the administration of antimicrobial agents is the most commonly used method. Pathogenic microorganisms are sensitive to one or more antimicrobial agents and at the same time are resistant to one or a number of conventional drugs (Delaat, 1975). Mastitis could be grouped, according to how the various infections respond to antibiotic therapy, into three groups.
Group I- Organisms that respond well to treatment (*Streptococcus agalactiae*)
Group II- Organisms that have variable responses (other *Streptococci*, *Staphylococci* and all Gram negatives.
Group III- Organism that are refractory to treatment (*Mycoplasma*, *Prototheca*, *Nocardia* and *Pasteurella*) (Woods, 1986).

Treatment of a cow acutely sick from mastitis must be directed towards saving the cow's life. All clinical cases should be treated as they occur, otherwise a permanent loss could commence. Before any attempt made to treat mastitis, selection of the most likely effective antibiotic for the treatment is essential. Antibiotics are selected according to the identified pathogen and sensitivity of the organism cultured from a milk sample. Sensitivity testing has advantages over blind treatment, in that, it helps to cure animals within short period of time and return to production, reduce further disease spread and serving as a source of infection, avoids the risk of bacteria developing resistance and is more of economical. Treatment of sick animals without sensitivity testing and indiscriminate drug usage by many non-professionals leads to the development of drug resistance. Conventional antibiotics like, penicillin, cloxacillin, erythromycin, and cephalosporins have excellent successes against mastitis caused by *Streptococcus*

agalactiae and *Str.dysgalactiae.* Before treating *Staphylococcus aureus* cases susceptibility testing is recommended. Systemic treatment with penicillin, ceftiofur or pirlimycin result greater cure when combined with local intramammary infusion containing cloxacillin and cephalosporin. Drugs like gentamycin, amikacin, trimethoprim-sulfa, and ticarcillin-clavulanic acid work against most coliforms, polymyxin B and cephalotin and tetracycline, ampicillin, neomycine and kanamycin work against 60-80% and 40-60% in *vitro*, respectively (Rebhun, 1995).

2.10 Drug Resistance

Approximately 70% of the antimicrobials used in dairy production are for treatment of clinical mastitis (Waldner, 2002). According to SVARM (2001) the uses of antimicrobials have increased by 37% from 1990 to 2000. The uses of antimicrobials have, overtime, increased the number of antimicrobial-resistant microbes globally, and any use of antimicrobial agents will to some extent benefit the development of resistant strains (Williams, 2000). The occurrence of antimicrobial resistance in microbes makes it more difficult to treat individual animals. Unnecessary or inappropriate usage (wrong dose, drug or duration) contributes the most to the increase in antimicrobial resistance without improving the outcome of treatment (Williams, 2000).

Due to one or other reasons bacterial agents that cause mastitis develop resistance of variable degree to different antibiotics. The emergence of bacteria resistance to antimicrobial agents within animal population or during therapy is a matter of great concern (Fraster, 1986). Drug resistance isolated from domestic animals is important in limiting the use of antimicrobial agents in animals and potentially in humans (Prescott and Baggot, 1988). Among the main pathogenic organisms causing mastitis, some *Streptococcus* species and *S. aureus* develop resistance to antibiotics like penicillin, streptomycin and oxytetracyclines (Ak, 2000). Some of the bacterial agents isolated from

a case of mastitis that develop resistance for in *vitro* trial in different places are summarized in table below.

Table 1: Some of bacterial isolates that develop resistance to some antibiotics

Bacteria	Type of Drug	% of resistance
Streptococcus dysgalactiae	penicillin, oxacillin, chloramphenicol	3.7
Stre. dysgalactiae	erythromycin and oxyteteracycline	7.4
Streptococcus uberis	penicillin, oxacillin, oxyteteracycline	2.6
Beta haemolytic streptococcus	oxyteteracyclin	1.9
Beta haemolytic streptococcus	penicillin, oxacillin, chloramphenicol, erythromycin	3.7
Staphylococcus aureus	penicillin	31.3
Eschrichia coli	oxytetracycline	26.1
Pseudomonas aeruginosa	oxytetracyclin	30.4
Corynebacterium isolates	penicillin, erythromycin	15
Corynebacterium isolates	chloramphenicol	20
Staphylococcus aureus	penicillin	83
Staphylococcus aureus	streptomycin	60

Source: (Ak, 2000; Heras *et al*., 1999; Mallikarjunaswmy *et al*., 1997; Woods, 1986; Kerro, 1997;Pankey 1989).

2.11 Public Health Importance Of Contagious Mastitis

Milk from mastitic cows may contain harmful pathogenic microorganisms to human beings. Bad milk would be responsible for more sickness and deaths (Howard, 1993). Although, pasteurization has eliminated the gross public health significance of milk, there

are still enough consumers of raw milk to mention the various mastitis or milk related factors affecting human health. There has also been reported of individuals taken ill after consuming milk products high in toxins produced by *Staphylococcus aureus* that pasteurization did not eliminate. Besides *Escherdichia coli* can cause enteritis, diarrhoea and vomiting (Woods, 1986).

Milk and other dairy products are frequently infected with S. *aureus*. According to Gilmour and Harvey (1990) milk of infected animals is the main source of enterotoxigenic *S. aureus* of animal origin. For example certain *S. aureus* strains produce heat-resistant enterotoxins, which cause nausea, vomiting and abdominal cramps when ingested by humans and are responsible for staphylococcal food poisoning outbreaks (Kluytmans *et al.,* 1997).

Toxins are produced due to improper cooling of milk, during cheese manufacture from raw milk and also due to post-processing contamination. These toxins cannot be destroyed by heating or drying (National Mastitis Council, 1996).

The bovine mammary gland can be a significant reservoir of enterotoxigenic strains of *S. aureus.* Two different types of toxin with super-antigen activity can be produced: enterotoxins and toxic shock syndrome toxin (TSST-1). The staphylococcal enterotoxins (SEs) have been divided into five serological types (SEA, SEB, SEC, SED, and SEE) on the basis of their antigenic properties (Dinges *et al*., 2000). The strains producing the staphylococcal enterotoxin type C (SEC) have been widely isolated from *mastitis*-afflicted cows (Matsunaga *et al*., 1993; Lee *et al.,* 1998; Cardoso *et al*., 1999).

In humans, *S. agalactiae* has been described as one of the most common factors of invasive infections in neonates, but it also causes invasive and non-invasive infections in adults (Schuchat, 2001). *S. agalactiae* also causes significant morbidity and mortality in humans, both infants and adults, all over the world (Blumberg *et al.,* 1992; Dargent-

Molina 1988). In neonates, S. *agalactiae* is mostly acquired from the mother's vagina in early-onset disease, although community and breast milk transmissions have been reported (Bingen *et al*., 1992). In adults, *S. agalactiae* occurs preferentially in certain individuals, such as diabetics, pregnant and post-partum women, and immune compromised patients, emphasizing the opportunistic nature of the infection (Lerner *et al.,* 1977). Furthermore, humans act as a significant reservoir of *S. agalactiae*, since these bacteria may be carried in the vaginas of women without apparent clinical signs (Huet *et al.,* 1993). Another public health concern regarding mastitis is antibiotic residues in milk due to extensive use of antibiotics in the treatment and control of the disease. Antibiotic residues in foods can lead to severe reactions in people allergic to antibiotics and, low levels; can cause sensitization of normal individuals and development of antibiotic-resistant strains of bacteria. Compliance with recommended withholding time helps minimizing the risk of antibiotic residues to occur in milk which is the producers' responsibility (White and Dermott, 2001).

3. MATERIALS AND METHODS

3.1 Study Area and Animals

The study was conducted in Jimma town of Oromia Regional State, south-western Ethiopia which is located at 355km South-Western of Addis Ababa. The area lies at 7°41'N latitude and 36°50'E longitude and has an altitude of 1704 meters above sea level. The area is characterized by a humid tropical climate of heavy annual rainfall that ranges from 1200-2000 mm. About 70% of the total annual rainfall is received during rainy season, which lasts from the end of May to early September. The mean annual maximum and minimum temperature ranges from 25°C-30°C and 7°C-12°C, respectively (OPEDJZ, 2002). A total of 58, 312 livestock populations are found in Jimma town. These include 10,000 cattle, 4,860 sheep, 1,680 goats, 451 horses, 156 donkeys, 35 mules, and 36,350 poultry (JTSSMIDAO, 2011).

3.2 Study Animals

The study animals include all cross breed lactating dairy cows managed in Jimma town. Dairy cows were kept as source of milk and yoghurt for the town. The average holding capacity per household was eight lactating cows(Range: 1 to16). All the cows in this study were hand milked except one farm and most of them milked two times a day during lactation period. The study population comprises of 206 crossbred lactating cows found in Jimma town. Most are kept indoors, and few graze in the field occasionally. Some herds also kept in both type systems. All are usually stayed in tie stalls provision of some supplementary diet in addition to the natural pasture. Smallholder dairy farm is increasingly becoming an important source of milk supplies to households as well as a means of income generation in urban and peri-urban areas of Jimma. Manure removal is made on a daily basis in all herds. Pre-milking and post-milking hygienic procedures such as udder washing and drying are not well practiced, but some farmers do it.

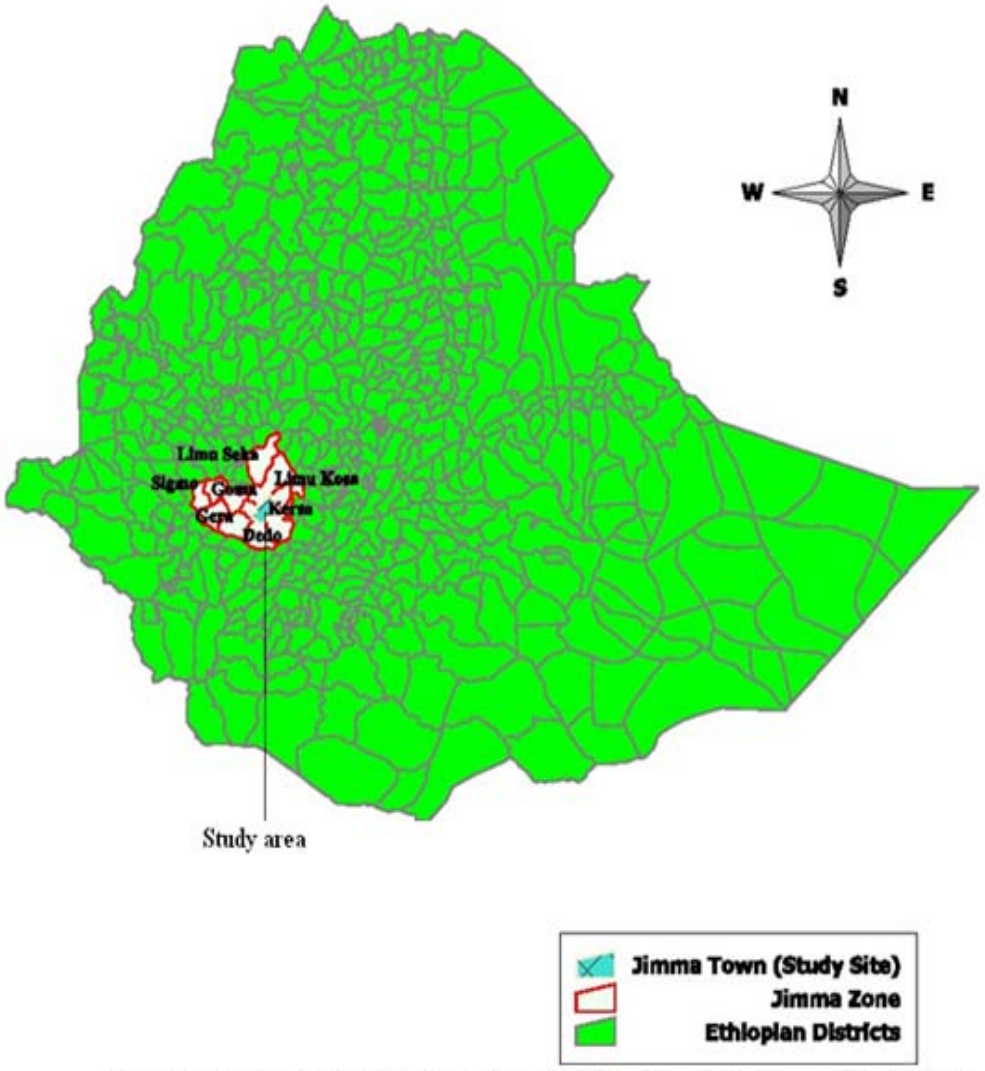

Fig. 1 Map Showing Ethiopian Administrative Districts and Study Site

3.2 Study Design

A cross-sectional study was conducted from October 2012 to May 2013.

3.3 Sampling Strategy

Milk samples were taken from 50 farms whose are found in Jimma town. All lactating cows in the 50 farms were included in the study.

3.4. Study Methodology

3.4.1. Data collection

Data regarding potential risk factors such as farm, cow (parity, lactation stage, hygiene scores, calf feeding, hand washing, cow and heifer purchased, source of water, drying udder, washing udder, type of antibiotic used, floor type, barn type) and quarter factor were collected from farm owners by questionnaires and farms visits. Age of the animals was categorized as adults (≤6 years) and old (>6). Stage of lactation was categorized as early (≤90 days), mid (90-180 days), and late (>180 to the beginning of dry period). Parity was categorized as few (with ≤ 2 calves), moderate (3–6calves) and many (>6 calves) (Megersa *et al.,* 2012). Milkers categorized as ≤ 3 milkers; 4-7 milkers > 7 milkers; herd size classified as < 20 cattle, 20-40 cattle, > 40 cattle; experience of dairying was categorized as 1-13 years, 14-26 years, 14-26 years. Cow udder, flank, tail and leg hygiene were assessed and scores were collected using a 4-point scale described previously (Schreiner and Ruegg, 2002). An udder hygiene score (UHS), Flank hygiene score (FHS), tail hygiene score (THS) or leg hygiene score (LHS) of 1 referred to no contamination of the skin of the rear of the udder or the hind limb between the hock and coronary band. A score of 2 was slightly dirty (2-10% of the area covered in dirt), a score of 3 moderately dirty (10-30% of the area covered in dirt), and a score of 4 indicated caked-on dirt (>30% of these areas completely covered in dirt).

Clinical inspection of the udder: The udder was first examined visually and then through palpation to detect possible fibrosis, inflammatory swellings, visible injury, tick

infestation, atrophy of the tissue, and swelling of supramammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Information related to the previous health history of the mammary quarters and causes of blindness was obtained from interviews with owners of the farm. Viscosity and appearance of milk secretion from each mammary quarter were examined for the presence of clots, flakes, blood, and watery secretions. The udder was also inspected for the presence of any grossly visible injury and ticks.

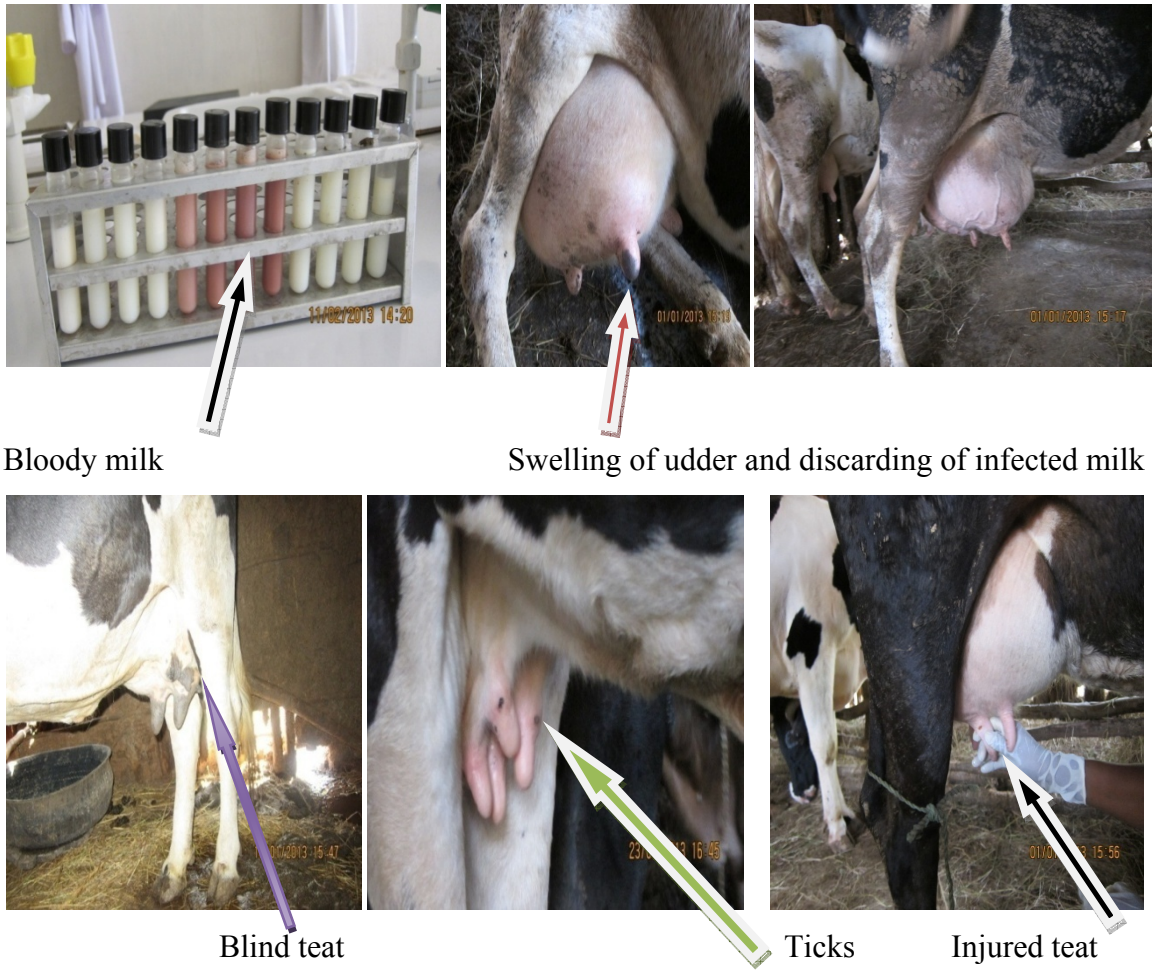

Figure 2. Clinical inspection of the udder and milk while sampling

Preparing udders and teats: Udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with cotton or gauze sponge moistened with 70% ethyl alcohol. In order to avoid contamination disposable gloves were worn throughout the sampling process. Recontamination of teats during scrubbing was avoided, the teats on the far side of the udder first, then those on the near side. A separate pledged was used for each teat. Scrubbing was continued until a new surface of the cotton remains clean.

Milk sample collection: The milk samples were collected according to the protocol described by National Mastitis Council (NMC, 1990). Strict aseptic procedures were used when collecting milk samples 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. Teats towards sample collect were taken first and then the far ones. The first 3-4 streams of milk were discarded. The collecting vial was as near horizontal as possible and by turning the teat to a near horizontal position, 10 mL of milk was collected into the vial. Samples were transported in ice box to Mastitis and Milk Quality Laboratory of the School of Veterinary Medicine, Jimma University College of Agriculture and Veterinary Medicine for analysis. In the lab it was stored at 4 °C for a night or immediately cultured.

Time of sample collection: Samples for culture were collected before milking that was most convenient under the management conditions of the individual cows.

3.4.2 CMT Screening

The CMT was used to diagnose the presence of subclinical mastitis following the procedures described by NMC (1999). A squirt of milk about 2ml from each quarter of the udder was placed in each of four shallow cups of CMT paddle cup and then an equal amount of the commercial reagent was added. A gentle circular motion was applied in a

horizontal plane for 15 seconds after which the positive samples shown gel formation within a few 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), 1 (weak positive), 2 (distinctive positive), and 3 (strong positive). Quarters with CMT score of 1, 2, and 3 were judged as positive. Cows were considered positive for CMT, when at least one quarter turned out to be positive for CMT and the interpretation is presented in (Appendix 2).

3.4.3 Bacteriological culture

Bacteriological isolation and identification were conducted according to the procedures of National Mastitis Council (NMC, 1999). Using sterile disposable culturing loop full, of milk was streaked onto one fourth of a plate blood agar. Supplemented with 7% defbrinated washed ovine blood and MacConkey agar and incubated aerobically at 37^{o}C for 24 - 48 h. Bacteria were identified using standardized procedures. Gram staining was performed (Atlas *et al.,* 1995) and Gram-positive cocci that occurred in clusters under the microscope were subjected to preliminary biochemical tests (the catalase and oxidase tests). For identification of *S. aureus* catalase-positive cocci were identified according to colonial morphology, haemolysis production, tube coagulase test with rabbit plasma, DNAse and polymyxin resistant tests. For identification of *Streptococcus agalactiae*, Catalase negative cocci were transformed to Edward's media for the detection of esculin hydrolysis and growth. CAMP test was conducted for esculin hydrolysis negative bacteria and CAMP test positives were considered as *Streptococcus agalactiae,* where as CAMP test negatives considered as other *Streptococci.*

3.4.4 Antimicrobial Susceptibility Testing

Antimicrobial sensitivity was conducted using agar disc diffusion method. Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. The bacteria used for this study were major isolates (*Staphylococcus aureus* and *Streptococcus agalactiae*) from mastitic quarters. For Streptococcus species and *C. bovis* blood was added to Mueller – Hinton agar. Two to four well-isolated colonies of the same morphological type were selected from the 7% sheep blood agar and suspension was made in a sterile saline. Turbidity of the bacterial suspension was adjusted by comparing with 0.05 McFarland turbidity standards. A sterile swab was dipped into the standardized suspension of the bacteria and excess fluid was expressed by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was streaked in three directions over the entire surface of the Mueller Hinton agar with the objective of obtaining a uniform inoculation and a final sweep with the swab was made against the agar around the rim of the petridish. The inoculated plates were allowed to stand for not more than 15 minutes and the discs were placed on the agar surface using sterile forceps. Each disc was gently pressed with the point of a sterile forceps to ensure complete contact with the agar surface (Quinn *et al*., 1999). Susceptibilities of the isolates to a panel of seven different antibiotic discs (6 µm in diameter, Mast group LTD Mersey Side, UK) were determined. For this study Ampicillin (33µg), Amoxacilline +CLAV (30+15 µg), Tetracycline (80µg), Trimethoprim +Sulfa (5.240µg), Tylocine (150 µg), Enrofloxacin (10 µg), Polymyxin (150 µg), Streptomycin (100µg) were used to compare their efficacy. Antibiotic discs were gently pressed onto the inoculated Mueller Hinton agar to ensure intimate contact with the surface and the plates were incubated aerobically at 37°C for 18 h – 24 h (NCCL; 1999). Inhibition zone diameters were measured and values obtained from the National Committee on Clinical Laboratory Standards (NCCL, 1999) were used to interpret the results obtained. *Staphylococcus aureus and S.agalactae* isolates were then classified as resistant, intermediate resistant or susceptible to a

particular antibiotic. Multiple antibiotic resistant (MAR) phenotypes were recorded for isolates showing resistance to three and more antibiotics (Rota *et al.,* 1996).

3.4.7 Data Analysis

The collected data was entered into Excel sheets and analysis was done using SPSS 2007, version 16. Descriptive statistics like percentage were used to calculate percentage of antimicrobial resistant, intermediate resistant and susceptibility pattern of the isolates of *S. aureus* and *S. agalactae* to different antimicrobial agents. Prevalence of mastitis related to specific risk factors was determined as the proportion of affected cows out of the total examined. Logistic regression analysis was used to measure the degree of association between risk factors. In all test applications, a probability level was $P < 0.05$ was considered statistically significant

4. RESULTS

A total of 206 lactating cows (cross breed) in Jimma town dairy farms were tested for presence of contagious mastitis. Out of 824 quarters examined 50 (6.06%) were found to be blind leaving 774 quarters were functional. Out of the total quarters examined, 404 (52.2%) were affected by CMT screening: 34 clinically and 370 sub clinically. The overall prevalence of contagious mastitis at cow level was 27.7%. From this 19.9% were *S. aureus* and 7.8% were *S. agalactae*. The overall prevalence of contagious mastitis at quarter level was 7.4%. From this 5.3% *S.aureus* and 2.1 were *S. agalactae*. Out of 404 infected quarters the proportion were: 92 (22.8) left front, 102(25.2) Left Rear, 111(27.5) Right rear R, 99 (24.5) Right front. All studied herds use neither dry cow therapy nor pre and post teat dipping mastitis control strategy. In addition, all herds practice hand milking except one which uses milking machine; all of them wash their hand before milking. None of herds checks cows or heifers for mastitis during purchasing or use CMT for screening of mastitis.

4.1 Herd level risk factors affecting prevalence of mastitis

At herd level risk factors, risks that were considered to affect prevalence of contagious mastitis were; calf feeding, herd size, experience of dairying, floor type, bedding, dry period, presence of maternity pens, source of water ,milkers, milking techniques, pre stripping, udder washing, drying udder, number of per cloth, teat disinfection, cow purchase and heifer purchased. Out of these factors the potential risks for the occurrence of contagious mastitis in this study were: floor type, source of water, milkers and heifer purchased ($p < 0.05$).

Table 2. Herd level risk factors of Mastitis Screened by CMT (*Staphylococcus aureus* and *Streptococcus agalactiae*)

Risk factors		N	CMT		OR	95%CI	P- value
			Negative	Positive			
Herd size	< 20 cattle	224	108(48.2%)	116(51.8%)	1		
	20-40 cattle	505	237(46.9%)	268(53.1%)	1.64	[0.267-9.989]	0.595
	> 40 cattle	45	25(55.6%)	20(44.4%)	0	[0.021-0.097]	0.996
Calf feeding	Bucket	589	251(42.6%)	338(57.4%)	1		
	Residual	185	119(64.3%)	66(35.7%)	0.07	[0.114-8.042	0.233
Experience	1-13 years	512	230(44.9%)	282(55.1%)	1		
	14-26 years	208	110(52.9%)	98(47.1%)	0.494	[0.155-1.576]	0.233
	> 26 years	54	30(55.6%)	24(44.4%)	0.958	[0.114-8.042]	0.968
Floor type	Concrete	559	289(51.7%)	270(48.3%)	1		
	Wood	193	73(37.8%)	120(62.2%)	3.947	[3.254-59.768]	0.000*
	Soil	22	8(36.4%)	14(63.6%)	1.004	[0.091-11.077]	0.997
Bedding	No	409	177(43.3%)	232(56.7%)	1		
	Yes	365	193(52.9%)	172(47.1%)	1.379	[0.382-4.984]	0.624
Dry period	1 month	42	16(38.1%)	26(61.9%)	1		
	2 month	111	62(55.9%)	49(44.1%)	0.063	[0.003-1.486]	0.087
	3 month	621	292(47.0%)	329(53.0%)	5.162	[0.200-133.4]	0.323
Presence of maternity	No	170	84(49.4%)	86(50.6%)	1		
pens	Yes	604	286(47.4%)	318(52.6%)	3.895	[0.790-19.212]	0.095
Source of water	Tape	424	189(44.6%)	235(55.4%)	1		

	River	91	42(46.2%)	49(53.8%)	21.043	[0.735-602.65]	0.075
	Spring	99	53(53.5%)	46(46.5%)	0.119	[0.016-0.876]	0.037*
	Well	160	86(53.8%)	74(46.2%)	10.731	[2.590-44.468]	0.001*
Milkers	≤ 3milkers	717	334(46.6%)	383(53.4%)	1		
	4-7 milkers	9	2(22.2%)	7(77.8%)	7.42	[7.216-1.599]	0.001*
	> 7 milkers	48	34(70.8%)	14(29.2%)	2.578	[0.164-40.589]	0.501
Milking	Squeezing	306	142(46.4%)	164(53.6%)	1		
Techniques	Stripping	468	228(48.7%)	240(51.3%)	0.434	[0.132-1.434]	0.171
Pres tripping	No	473	212(44.8%)	261(55.2%)	1		
	Yes	301	158(52.5%)	143(47.5%)	0.251	[0.068-0.921]	0.037
Udder washing	No washing	88	25(28.4%)	63(71.6%)	1		
	Whole udder	655	318(48.5%)	337(51.5%)	-	-	0.996
	Wash teat only	31	27(87.1%)	4(12.9%)	-	-	0.995
Drying udder	No	410	177(43.2%)	233(56.8%)	1		
	Yes	364	193(53.0%)	171(47.0%)	-	-	-
Number of per cloth	No drying	402	193(48.0%)	209(52.0%)	1		
	Separate towel	202	108(53.5%)	94(46.5%)	-	-	0.997
	Shared towel	170	69(40.6%)	101(59.4%)	-	-	-
Teat disinfect	No	375	173(46.1%)	202(53.9%)	1		
	Yes	399	197(49.4%)	202(50.6%)	0.733	[0.218-2.469]	0.617
Cow purchase	No	636	277(43.6%)	359(56.4%)	1		
	Yes	138	93(67.4%)	45(32.6%)	0.013	[0.003-0.053]	0..96
Heifer purchased	No	603	301(49.9%)	302(50.1%)	1		

	Yes	171	69(40.4%)	02(59.6%)	5.4	[21.843-609.7]	0.000*

There was a significant difference (P<0.05) on the prevalence of mastitis between cows kept under different floor type. Cows kept on wood and soil floor had higher prevalence of contagious mastitis than cows kept on concrete floor type and this was significantly difference (P<0.05). Cows kept on wood floor type had higher odds of contagious mastitis as compared to cows kept on concrete. (OR=3.947, CI=3.254-59.768) (Table. 2). Herds used well water source for washing are more likely affected by contagious mastitis as compared to herds used tap water. There is also a significant difference between the numbers of milkers. The prevalence of contagious mastitis is high in herds with many milkers (P<0.05) (OR=7.42, CI= [7.216-1.599]).There is also significant difference in purchased heifer with those not purchased heifer. The prevalence of contagious mastitis were high in purchased heifer when compared to that not purchased heifer (OR=5.4, CI= [21.843-609.7]).

4.2 Cow level risk factors affecting prevalence of mastitis

At cow level risk factor risks that considered affecting prevalence of contagious mastitis were; age, lactation stage, parity, udder hygiene status, flank hygiene status, leg hygiene status and tail hygiene status (Table.3). In this study all the cow level risk factors were not statistically significant (P>0.05).

Table 3. Cow level risk factors of mastitis Screened by CMT *(S. aureus* and *S. agalactae*)

Risk factors		N	CMT		OR	95%CI	P- value
			Negative	Positive			
Age	≤6 years	185	86(46.5%)	99(53.5%)	1		
	>6 years	589	284(48.2%)	305(51.8%)	2.008	[0.671-6.008]	0.212
Lactation stage	Early (≤90 days)	276	155(56.2%)	121(43.8%)	1		
	Mid(90-180days)	267	129(48.3%)	138(51.7%)	1.441	[0.690-3.008]	0.33
	Late (>180 days)	231	86(37.2%)	145(62.8%)	2.07	[0.949-4.516]	0.067
Parity	Few(≤2)	437	211(48.3%)	226(51.7%)	1		
	Moderate(3-6)	256	126(49.2%)	130(50.8%)	1.22	[0.566-2.628]	0.611
	Many(>6)	81	33(40.7%)	48(59.3%)	1.357	[0.274-6.730]	0.709
Udder hygiene status	1	40	19(47.5%)	21(52.5%)	1		
	2	511	245(47.9%)	266(52.1%)	0.591	[0.089-3.935]	0.586
	3	176	89(50.6%)	87(49.4%)	0.233	[0.032-1.695]	0.15
	4	47	17(36.2%)	30(63.8%)	1.362	[0.144-12.87]	0.788
Flank hygiene status	1	28	17(60.7%)	11(39.3%)	1		
	2	4249	186(43.9%)	238(56.1%)	0.085	[0.002-3.925]	0.207
	3	203	101(49.8%)	102(50.2%)	0.023	[0.000-1.063]	0.054
	4	119	66(55.5%)	53(44.5%)	0.053	[0.001-2.392]	0.13
Leg hygiene status	1	20	13(65.0%)	7(35.0%)	1		
	2	479	225(47.0%)	254(53.0)%	12.692	[0.296-544.39]	0.185
	3	181	84(46.4%)	97(53.6%)	31.588	[0.623-1.6013]	0.085
	4	94	48(51.1%)	46(48.9%)	22.27	[0.383-1.2943]	0.134
Tail hygiene status	1	12	4(33.3%)	8(66.7%)	1		
	2	463	232(50.1%)	231(49.9%)	1.152	[0.033-40.789]	0.938
	3	200	83(41.5%)	117(58.5%)	1.524	[0.044-53.235]	0.816
	4	99	51(51.5%)	48(48.5%)	0.751	[0.019-29.959]	0.879

1= no contamination, 2= slightly dirty, 3= moderately dirty, 4= dirty

4.3 Quarter Level Risk Factors Affecting Prevalence of Mastitis *(S. aureus* and *S. agalactae*)

Risks that were considered to affect prevalence of contagious mastitis at quarter level were quarter position, teat injury and presence of ticks (Table. 4). All the risk factors at quarter level were not statistically significant for the occurrence of contagious mastitis in this study (P>0.05).

Table 4. Quarter level risk factors of mastitis by CMT screening test.

Quarter level risk factors		N	CMT		OR	95%CI	P-value
			Negative	positive			
Quarter Position	LF	192	100(52.1%)	92(47.9%)	1		
	LR	193	91(47.2%)	102(52.8%)	1.143	[0.577-2.264]	0.701
	RR	193	82(42.5%)	111(57.5%)	1.209	[0.614-2.381]	0.583
	RF	196	97(49.5%)	99(50.5%)	1.052	[0.528-2.095]	0.886
Teat injury	No	55	28(50.9%)	27(49.1%)	1		
	Yes	719	342(47.6%)	377(52.4%)	0.347	[0.068-1.777]	0.447
Presence of ticks	No	83	42(50.6%)	41(49.4%)	1		
	Yes	691	328(47.5%)	363(52.5%)	0.347	[0. 068-1.777]	0. 204

LF=Left front, LR= left rear, RR= Right rear, RF=Right front

Table 5. Summary of potential risk factors for occurrence of contagious mastitis (*Staphylococcus aureus* and *Streptococcus agalactiae*) by logistic regression

Risk factors		N	CMT Test result		OR[95%CI]	P-value
			negative	positive		
Floor type	Concrete	559	289(51.7%)	270(48.3%)	1	
	Wood	193	73(37.8%)	120(62.2%)	3.143 [1.382, 3.321]	0.001*
	Soil	22	8(36.4%)	14(63.6%)	3.326 [1.160, 9.534]	0.025*
Source of	Tape	424	189(44.6%)	235(55.4%)	1	
Water	River	91	42(46.2%)	49(53.8%)	21.043[0.735-602.65]	0.075
	Spring	99	53(53.5%)	46(46.5%)	0.119 [0.016-0.876]	0.037
	Well	160	86(53.8%)	74(46.2%)	10.731[2.590-44.468]	0.001*
Heifer	No	603	301(49.9%)	302(50.1%)	1	
purchased	Yes	171	69(40.4%)	102(59.6%)	5.650[3.276-9.745]	0.000*
Lactation	≤90 days	276	155(56.2%)	121(43.8%)	1	
stage	90-180 days	267	129(48.3%)	138(51.7%)	2.16[1.511-3.087]	0.000*
	>180 days	231	86(37.2%)	145(62.8%)	1.576[1.101-2.256]	0.013*
milkers	≤3	717	334(46.6%)	383(53.4%)	1	
	4-7	9	2(22.2%)	7(77.8%)	7.76 [2.970- 96.831]	0.001*
	>7	48	34(70.8%)	14(29.2%)	0.902[.419-1.940]	0.791
Total		774	370(47.8%)	404(52.2%)		

4.4. Culture Result

The prevalence of mastitis by CMT was 52. %and by culturing was 62.1%. The CMT detects 404 positive results from the 774 quarters .But by culturing 481 bacterial were obtained (Table 6).

Table 6. Result of diagnosis of mastitis by CMT and Culturing method

Diagnostic tool		Frequency	Percentage
CMT test result	negative	370	47.8
	positive	404	52.2
	Total	774	100.0
Culture result	negative	289	37.3
	positive	481	62.1
	Contaminated	4	0.5
	Total	774	100.0

4.5. Bacterial Isolates

The list, number and proportion of the bacterial isolates from a total of 206 cows (774 quarters) are presented in Table 7. The isolates from the clinical mastitis and sub clinical mastitis were, *S. aureus* 41(19.9%) and *S.agalactae* 16 (2.06%).

Table 7. Microbial agents isolated from clinical and sub clinical mastitis (contagious mastitis cases Jimma town herds from October 2012 to May 2013

Isolates	**N**	**No positive**	**Prevalence**
S. aureus	774	41	19.9
S.agalactae	774	16	7.8
Total	**774**	**57**	**27.7**

N=Total number

4.6. Antimicrobial Susceptibility Test Results

A total of 57 isolates (41 (19.9%) *S. aureus and 16 (2.06%) S.agalactae) were* tested for susceptibility to nine antimicrobials commonly used for treatment of mastitis. Responses of the organisms to antibiotics tested were summarized in table 8. There were wide ranges of variations in the sensitivity patterns of the isolates to antimicrobial agents tested. The antibiotics were Ampicillin (33µg), Amoxicillin +CLAV (30+15 µg), Tetracycline (80µg), Trimethoprim +Sulfa (5.240µg), Tylocine (150 µg), Polymyxin (150 µg), Streptomycin (100µg), Ampicillin (33 µg), and Enrofloxacin 10 µg. Out of 41S.aurus isolates 36 (87.8%) were found to be resistant to oxacillin after screening by oxacillin resistance screening agar base.

Table 8. Percentages of in vitro susceptibility to selected antimicrobial agents for *S.agalactiae* and *S. aureus* isolates.

Drugs name	Categories	Spps. of bacteria		Total
		S .aureus	*S. agalactae*	
Amoxicillin +	susceptible	6(14.6%)	4(25.00%)	10(17.5%)
CLAV	resistant	35(85.4%)	12(75.0%)	47(82.5%)
Cefquinome	susceptible	38(92.7%)	16(100.0%)	54(94.7%)
	intermediate	1(2.4%)	0	1(1.8%)
	resistant	2(4.9%)	0	2(3.5%)
Streptomycin	susceptible	26(63.4%)	16(100.0%)	42(73.7%)
	intermediate	11(26.8%)	0	11(19.3%)
	resistant	4(9.8%)	0	4(7.0%)
Tetracycline	susceptible	18(43.9%)	2(12.5%)	20(35.1%)
	intermediate	9(22.0%)	4(25.0%)	13(22.8%)
	resistant	14(34.1%)	10(62.5%)	24 (42.1%)
Tylosine	susceptible	34(82.9%)	10(62.5%)	44(77.2%)
	intermediate	7(17.1%)	6(37.5%)	13(22.8%)
Trimethoprim +	susceptible	28(68.3%)	16(100.0%)	44(77.2%)
sulfa	intermediate	11(26.8%)	0	11(19.3%)
	resistant	2(4.9%)	0	2(3.5%)
Polymyxin	intermediate	0	10(62.5%)	10(17.5%)
	resistant	41(100.0%)	6(37.5%)	47(82.5%)
Ampicillin	susceptible	-	9(56.2)	9(56.2)
	intermediate	-	4(25%)	4(25%)
	resistant	-	3(18.8)	3(18.8)
Enrofloxacin	susceptible	-	13(81.2)	13(81.2)
	resistant	-	3(18.8)	3(18.8)
Total		41(71.9)	16(28.1)	57(100)

Cefquinome and Tylocine were the most effective antibiotics where 82.9 and 92.7% susceptibility were recorded, respectively. This was followed by Trimethoprim +Sulfa and streptomycin where effective for 68.3 %and 63.4 % respectively among the total isolates of *S. aureus.* Tetracycline and Amoxacilline were also effective against 43.9% and 14.6% of the total isolates of *S. aureus,* respectively.

All isolate of *S.aurues* was 100% resistant to Polymyxin B. 2.4% of Cefquinome, 26.8% of streptomycin, 22% of tetracycline, 17.1% of Tylocine, 26.8% of Trimethoprim +sulfa were intermediately resistant for all isolates of *S. aureus*.

When comparing the overall efficacy (on all isolates of *S.agalactae*) Cefquinome, Streptomycin (100µg), Trimethoprim +Sulfa are 100 % effective on of the total isolates of *S.agalactae.* Followed by Enrofloxacin, (81.2%), Ampicillin (56.2%), Tylocine (62.5%) and Amoxyciline+CLAv (25.0%) were susceptible to all isolates of *S.agalactae*. The least effective drug was tetracycline where only 12.5% of the total bacterial population was susceptible. In this study, Cefquinome, Tylocine and Trimethoprim +Sulfa were effective on *S. aureus* isolates. Cefquinome, Streptomycin and Trimethoprim +Sulfa were the most effective for all isolates of *S. agalalatae* which is 100% susceptible. From *S. agalactiae* Amoxicillin +CLAV and Tetracycline showed highest resistance (75%) and (62.5%) respectively.

(1.8%) of Cefquinome, (19.3%) of Streptomycin, (22.8%) of Tetracycline, (22.8%) of Tylosine, (19.3%) of Trimethoprim + sulfa and (3.5%) polymyxin were intermediate for contagious mastitis pathogen (*Staphylococcus aureus* and *Streptococcus agalactiae***)** Amoxicillin +CLAV (82.5%), tetracycline, (42.1%), and Polymyxin (82.5%) were resistant for both *S. aureus* and *S.agalactae,* respectively. This may be due prolonged use of this medicine in this town (Jimma).

5. DISCUSSION

Nowadays, the economic impact of clinical and subclinical mastitis is high in dairy industry. Losses occur from decreased milk production, treatment and labor costs, veterinary fees, risk of culling or death of the cow, and reduced milk quality and milk price (Nielen *et al.,* 1992; Durr *et al.*, 2008). Furthermore, low quality milk can consist of pathogens and their toxins, which may to hazardous for human health (Kasikci *et al.,* 2012).

Subclinical mastitis, which is hidden form of mammary gland infections, is a very complex disease while the causative agents were numerous. Contagious pathogens, such as *S. aureus* or *S. agalactiae,* which are the most common agents' related to subclinical mastitis, are responsible for the strong indicators of the presence of intramammary infections in the herd (Behiry *et al.,* 2012; Merl *et al*., 2003). In the present study, the overall prevalence of contagious mastitis at cow level was 27.7% in crossbred using CMT (19.9% *S.aureus* and 7.8% *S.agalactae*). The prevalence of contagious mastitis at quarter level was 7.36 % 5.29% *S. aureus* and 2.1% *S.agalactae*).

The overall prevalence of mastitis observed in this study was comparable to the results of previous findings in other parts of the country (Zerihun *et al*., 2013). In the work of Zerihun *et al. (*2013) the predominant organisms isolated from clinical and sub clinical mastitis is *Staphylococcus aureus* (28.7%) followed by *Streptococcus agalactiae* (21.2%). The predominance and primary role of *S. aureus* isolates in bovine mastitis has also been reported in other studies (Atyaib *et al*., 2006; Fadlelmoula *et al.,* 2007; Mekbib *et al.,* 2010). Again concerning the isolation rate of *S. aureus* (19.9%) in this study were greater than the findings of Bishi (1998) and Hussein (1999) who reported 9% and 10.69% prevalence in Addis Ababa, respectively. However, the present finding was lower than that of Workineh *et al*. (2002) and Kerro and Tareke (2003) where *S. aureus* accounted for 39.2% and 40.5% of the isolates, respectively, in their study at

Addis Ababa and southern Ethiopia. This variability in prevalence of mastitis between different reports could be attributed to differences in farm management practices or differences in study methods or differences in sample collection and transporting method.

The isolation rate of *S. agalactae* (7.8%) in this study was closely comparable with the findings of Alema 2008 (8.15%) in Bahir Dar milk shed., Kerro and Tareke (2003) who reported isolation rates of 13.1% *S.agalactiae*; but Bishi (1998) reported higher isolation rate (27%) for *Str. Agalactiae and* GodkinanLeslie (1990) and (Bartlett *et al.*, 1992 in Indiana (7.6%) and Ohio (10.2%) dairy farms, respectively.

In general *Staphylococci* species were the predominant isolates and this agrees with the findings of Buragohain and Dutta (2000) and Bhattacharya (2002), Zingser *et al*. (1991) in a survey conducted in Jamaica, the most common bacteria isolated were *Staphylococcus aureus* (27%). In India, Barbuddhe *et al*. (2001) reported 23.25% and 11.6% *Staphylococcus aureus* and *Streptococcus* species, respecively. Haile (1995) found 38.8% of *Staphylococcus aureus* isolates from milk samples as a dominant isolate and 6.8% of *Streptococcus* species.

The relatively high prevalence of *S. aureus* in this study could be associated with total absence of dry cow therapy and post milking teat dipping, the invariably hand milking practice, low culling rate of chronically infected cows (culling was usually due to feed shortage, aging and reproductive problem) and limited knowledge of farmers on segregation as a control option. The primary reservoir of contagious pathogens including *S. aureus* is infected quarter and the exposure of uninfected quarter is limited to the milking process (Fox and Gay, 1993).

In the present study higher prevalence of *S.aureus* than *S.agalactae* were recorded. This could be due to different factors mentioned by many scholars. Radostits *et al.* (2007)

asserted that *S. aureus* is well adapted to survive in the udder and usually establishes a mild sub clinical infection of long duration from which it shed in milk facilitating transmission to healthy animals mainly during milking. Globally *S. agalactiae* is a low prevalence pathogen. In Canadian bulk milk, its prevalence ranged between 6% in Alberta (Schoonderwoerd *et al.*, 1993), and 43% in Quebec (Guillemette *et al*., 1992). Furthermore, a study recently performed in Canada (Riekerink *et al*., 2010) demonstrated the low prevalence of *S. agalactiae* at 4.4% and in Argentina, the mastitis prevalence due to *S. agalactiae* has been 0.3% in the four quarters before-delivery (Calvinho *et al*., 2005).

Contagious mastitis highly prevalent in wood floor type when compared with that of concrete and soil floor type. This could be related with the level of that wood i.e hold water which enhance the proliferation of bacteria in that area. The hygiene of the farm and the animals itself also has a contribution for the survival of the bacteria; bad hygiene favours multiplication of the organisms like flies. The contagious bacteria which can be transmitted by flies from infected animal to non infected one. In other work Higher prevalence of mastitis reported in cows maintained in crackled concrete and muddy soil floor where manure and wet bedding were not frequently removed (Mekibib *et al.,* 2010). The association between soil floor and high prevalence of mastitis recorded in our study is consistent with the findings of Abera *et al.* (2010). The cows remained on the floor all day and got dirty. The floor was muddy, and drainage was difficult to maintain. In addition, the warm temperature and high humidity favoured the growth of organisms (Fox *et al.,* 1995).

The prevalence of contagious mastitis is high in herds purchasing heifers for replacement than those not purchasing. The most probable reason could be most of the time the farmers sell the cow and heifers with health problem and low production and buyers purchasing them without testing for udder problem. Research in several countries has demonstrated that up to 50% of purchased cows have subclinical infections Philpot

et al. (1978). It is better to buy only heifers (heifers generally do not have mastitis) or produce your own replacement animals.

Mastitis was highly prevalent in well water source when compared to that of tape, river and spring water sources. This is because water source can contaminate in many cases. Especially when the contaminated materials washed around water source and the waste material enter in water. The contaminated water source has many chances to increase prevalence of the diseases. There was chance to contaminate milkers hand, cow's udder and teat while washing and drying with towels. All these contaminated materials contribute to increase the prevalence of contagious mastitis. According to Flowerday (1998) and Hubble (1990) as the water passes through the atmosphere, over the surface of land and through the soil it may change in quality in many ways. It collects physical impurities (sediment, turbidity, organic matter), mineral impurities (hardness, alkalinity, iron) and biological impurities (algae, micro-organisms and bacteria). Impurities can cause problems with the performance of chemicals used in dairy hygiene and mastitis control.

The significant effect of stage of lactation on prevalence of contagious mastitis in this study was also reported by Nesru (1999), Mungube *et al.* (2004), Kerro and Tareke (2003) and Biffa *et al.* (2005) in Ethiopia. The former two authors reported higher prevalence of sub-clinical mastitis for cows in mid and late stage of lactation as it is the case in this finding, while the later two reported higher prevalence in early stage of lactation. The variations in the effect of stages of lactation between the different studies could be related probably to the disparities in age, parity and breed of the sampled animals.

The high occurrence of mastitis-induced blind mammary quarters, which has a direct influence on milk production with a subsequent impact on food security, signifies the importance of the problem. Lack of screening and treatment of subclinical mastitis and

inadequate follow-up of clinical and chronic cases coupled with persistent challenges of the mammary glands by microbial pathogens could be the main predisposing factors to quarter blindness. This hidden and gradual destruction of the mammary tissues would end with non-functional quarters. Though estimation of productivity losses incurred by mastitis is beyond the scope of this study, it may not be difficult to imagine the losses given the high proportion of nonfunctional quarters

In this study, *S. agalactiae* were found be resistant to amoxacilline (75%), tetracycline 62.5%), and polymyxin 6 (37.5%), whereas *S. aureus* were detected to be resistant to amoxicillin (85.4%), streptomycin (9.8%), tetra (82.9%) and polymycin (100%) resistant. The most effective antibiotics against to *S. agalactiae* were streptomycin (100%), cefquinome (100%), trimethoprim+sulfa (100%) while *S.aureus* was against to trimethoprim+ sulfa (68.3%), cefquinome (92.7 %%), and tetracycline (43.9%).

In present study (18.8 %) of ampicillin and (62.5%) of tetracycline were resistant to *S .agalactae* isolates. This finding were comparable with the finding of Gonzalo (2009) in Egypt which was 6.7% of ampicillin and 53.3% of tetracycline were resistant.

In the present study tetracycline (75%) is resistant to *S .agalactae* isolated. Similar result was recorded from Egypt by Jake *et al.* (2013). This observation is supports the findings of Biffa *et al.* (2005). Again the resistance of S.*aureus* to amoxicillin were recorded by Abera *et al.,* 2010 which is 36.1%

The high resistance of polymyxin B. (100%) in this study was similar with finding of Getahun *et al.,* (2008) at central of Ethiopia which (97.7%) and Trimethoprim, Polymyxin and Tetracycline demonstrated poor activity against 60%, 73% and 40%of *S.aureus* strains respectively. It was also recorded by Biffa *et al.,* (2005). However, it was generally reported that drug resistant strains of Staphylococci appeared to be increasing from time to time with varying rate of incidence (Blood and Radostits, 1989).

In the present study the highest resistant of *S. aureus to* Oxacillin were in agreement with the finding of Adebayo and Johnson 2006 which is 100% resistant to Oxacillin in South Africa. Failure of therapy may be due to a variety of factors the most common of which are: incorrect identification of the causative pathogens, mishandling and irrational application of antibiotics towards the control of mastitis (under-dosage, adulteration, inappropriate route and frequency of application, use of expired drugs and mixing with other chemicals) seems to have favored development of drug resistance and hence failure (Cruickshank, 1968).

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6. CONCLUSION AND RECOMMENDATION

The present study revealed that contagious mastitis (*S. aureus and Streptococcus spp.)* were prevalent in Jimma dairy farms. Again, the study confirms that the subclinical form is the most prevalent. Contagious mastitis prevalence was associated with several risk factors such as floor type, source of water, milkers, lactation stage and heifer purchased. The study also concludes *Staphylococci* and *Streptococci* are the most important causes of bovine mastitis, especially subclinical mastitis, in dairy farms. Culling of old and chronically affected cows, screening of cows and milk for clinical and subclinical mastitis, dry cow therapy, hygiene at milking and cow house hygiene should be considered in attempts to reduce prevalence of contagious mastitis. Using a simple screening test like CMT, farmers should test the dairy animals before purchasing; if possible positive animals should not be purchased. Moreover, extension services and training programs aiming at creation of awareness about the importance and prevention of contagious mastitis among dairy farmers is recommended.

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7. APPENDICES

Appendix 1.Questionaire survey on study of mastitis

Date……. Code no.

A. herd information

1. Farm structure

Farm owner……..

Address……

Location……..

2. General management

Herd size and composition of the farm.

Type of cattle	Number of animals	Breed(Local/Cross)
Lactating cow		
Pregnant cow		
Dry cow		
Heifers		
Bulls		
Calves		

What type of cal feeding do you use for your animals?

a) Residual suckling

b) Bucket feeding

For how long do you raise dairy cattle/ farming……..…

Grazing

a) Indoor

b) Out door

c) Both

Barn type

a) Concrete

b) Wood

c) Earth

Bedding

a) Straw/saw dust

b) No straw/Saw dust

Sleeping area for cows

a) Same as feeding

b) Separate area

Animal tethered while in house

a) Yes

b) No

Dry cow therapy

a) All cows, all quarters

b) Some cows, all quarters

c) Some quarters

c) None

Length of dry cow period……

Presence of Maternity room

a) Yes

b) No

Washing of milker's hand before milking

a) Yes

b) No

Source of water for hand washing

a) Tape

b) Well

c) River

Number of milkers in last week in the parlor……..

Milking techniques

a) Five finger squeezing=Y/N

b) Stripping=Y /N

c) Pre-stripping=Y/N

d) Teat dipping (pre/Post-milking teat dipping) =Y/N

e) Udder washing before milking=Y/N

a) teat only

b) The whole udder

f) Drying of udder before milking=Y/N

g) Type of cloth for drying

a) Separate towel

b) Shared towel

h) Number of cows per cloth……

i) Use of disinfectant in water =Y/N

j) access to fresh feed and water immediately after milking=Y/N

k) Mastitic cow milking last or stay in separate unit=Y/N

l) Treat all clinical mastitic cases with antibiotics=Y/N.

m) teat disinfection=Y/N

Cow purchased in last year

a) Yes

b) No

Heifer purchased in last year

a) Yes

b) No

Take milk sample of purchased cows

a) Yes

b) No

Preventive antibiotic treatment for heifers before caving

a) Yes

b) No

Availability of California mastitis test (CMT) on farm for SCM diagnosis

a) Yes

b) No

How often is CMT used?

a) Yes

b) No

Take milk samples for bacterial culture=Yes/No

B. Cow information of selected cows

No.	Cow identification	Body score(1-5)	Age	Lactation stage	Breed	Parity status	Calving interval	Hygiene score
1								
2								
3								
4								
5								
6								

C .Quarter information

Cow	Quarter	Clinical mastitis	Teat injury	Presence of ticks
	LF	Y/N	Y/N	Y/N
	LR	Y/N	Y/N	Y/N
	RR	Y/N	Y/N	Y/N
	RF	Y/N	Y/N	Y/N

7.3 Do you take milk samples (for bacteriological analysis) regularly in order to detect cows that are suffering from subclinical mastitis? Yes____ No____

Appendix 2. Interpretation of CMT findings
Source: Quinn *et al*. (1999)

From each quarter of the udder, a squirt of milk sample was placed in each of the cups on the CMT paddle and an equal amount of 3% CMT reagent was added to each cup and mixed well. Reactions were graded as 0 and Trace for negative, +1, +2 and +3 for positive (NMC1990; Quinn et al., 1999).

Score	Interpretation	Visible reaction
0	Negative	Milk fluid and normal
T(Trace)	Trace	Slight precipitation
1	Weak positive	Distinct precipitation but no gel formation
2	Distinct positive	Mixture thickens with gel formation
3	Strong positive	Viscosity greatly increased .strong gel i.e. cohesive with a convex surface

Appendix 3. Biochemical tests

Catalase test:

This demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. A drop of 3 % hydrogen peroxide poured on the glass slide and then small amount of the culture to be tested is picked from a nutrient agar with a clean sterile platinium loop or a clean, thin glass rod and this is added into hydrogen peroxide solution held on glass slide. The production of gas bubbles indicates a positive reaction. It occurs almost immediately. A false positive reaction may be obtained if the

culture medium contains catalase (ex. blood agar) or if an iron wire loop is used (Collee, 1989).

Oxidase test:

This test depends on the presence of oxidases in the bacteria that will catalyse the transport of electrons between electrons donors in the bacteria and a redox dye - tetramethyl-p-phenylene-diamine. The dye is reduced to a deep purple colour. The dye is used for screening species of *Alcaligenes, Pseudomonas, Flavobacterium and Pasteurella* spp, which give positive reactions and for excluding the Enterobacteriacae, all species of which give negative reactions.

Wet filter paper method:

A strip of filter paper is soaked with a little freshly made 1 % solution of the reagent and then at once used by rubbing a speck of culture on it with a platinum loop. A positive reaction is indicated by a dark purple colour appearing with in 10 seconds, a delayed positive reaction by colouration in 10 to 60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds (Quinn, *et al*., 1999).

Tube coagulase test):

0.5 ml of rabbit plasma is placed in a small test tube. Two drops of a heavy suspension made from the culture on an agar plate in sterile water, are added then incubated at 37oC. A positive test with cloting of the plasma can occur in 2 to 4 hrs. However, many weak coagulase positive strains will coagulate the plasma only after overnight incubation.

Appendix 4. Media used

1. **Blood agar base** (Oxoid,CM0271 Basingstoke, Hampshire, England) Composition (g/l): Nutrient substrate (heart extract and peptones) 20.0; sodium chloride 5.0; agar-agar 15.0.

Preparations

Forty grams was suspended in 1litre of deminiralized water by heating in a boiling water bath and autoclaved at 1210c for 15 minutes. Cooled to 45-500C and 5-8% sterile defibrinated blood was added and mixed taking care to avoid bubble formation. Poured to plates. Ph 6.8 + 0.2 at 250C.

2. Mac Conkey (500g), Oxoid CM 0007Basingstoke, Hampshire, England.

Composition (g/l): Peptone from casein 17.0; peptone from meat 3.0; sodium chloride 5.0; lactose 10.0; bile salt mixture 1.5; neutral red 0.031; crystal violet 0.001; agar-agar 13.5.

Fifty grams was Suspended in 1litre of demineralised water by heating in boiling water bath and autoclaved for 15 minutes at 1210C. Ph 7.1+ 0.2.

3. Edwards medium (modified) 500g, Oxoid, Basingstoke, Hampshire, England.

Composition (g/l): 'Lab-Lemco' powder 10.0; peptone 10.0; asculin 1.0; sodium chloride 5.0; crystal violet 0.0013; thallous sulphate 0.3; agar 15.0.

Preparation

Forty-one grams of the media was suspended in 1liter of distilled water. Brought to the boil to dissolve completely. Sterilized by autoclaving at 1150C for 20 minutes. Cooled to 500Cand 5-7% of sterile sheep blood was added and mixed well and poured to plates. Ph 7.4+ 0.2.

4. Oxacillin Resistance Screening Agar Base M1454,CM 1008 Basingstoke, Hampshire, England

Composition

Ingredients	Gms / Litre
Peptic digest of animal tissue	11.800
Yeast extract	9.000
Mannitol	10.000
Sodium chloride	55.000
Lithium chloride	5.000
Aniline blue	0.200
Agar	12.500
Final pH (at 25°C)	7.2±0.2

Direction

Suspend 51.75 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool at 45-50°C and aseptically add rehydrated contents of 1 vial of Oxacillin Resistance Selective Supplement (FD191). Mix well and pour into sterile Petri plates. Caution: Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

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Once the swab sample is taken you just need to streak the prepared Oxacillin Resistance Agar plate near the edges and then across the plate by the diminishing sweep technique. The inoculated plates should be incubated at 37ºC for 24 hours. The colonies of MRSA should have a dark blue colour. To confirm the blue colonies you may perform a coagulase test.

The typical colonies which showed an **intense blue in colour** on a colourless background of ORSAB media were oxacillin resistance strain of *S.aureus*, while on the other half of the petridish the colonies showed no changing of the color of ORSAB media, so the srain on that side were not Oxacillin resistance (Barrett *et al.,* 1986).

5. Deoxyribonuclease Test Agar (DNase Test Agar),Oxoid CM032 Basingstoke, Hampshire, England).

Deoxyribonuclease Test Agar is recommended for the detection of deoxyribonuclease activity of bacteria and fungi, and especially for identification of pathogenic Staphylococci.

Composition:

ingredients	Grams/Litre
Tryptose	20.0
Deoxyribonucleic acid	2.0
sodium chloride	5.0
agar	15.0

pH 7.3 +/-0.2 at 37°C

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Appearance: Faintly beige colored, homogeneous, free flowing powder.

Gelling: Firm

Color and Clarity: Slightly brownish-yellow colored, clear to slightly opalescent gel forms in petri plates.

Directions:

Suspend 42 g in 1 litre of distilled water and heat to the boiling and constant stiring to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour it into the plates.

Bacteria are streaked on to the surface of the agar medium and incubated. After inoculation and 18-24 hours incubation the growth on the surface of the agar is flooded with 1N hydrochloric acid. Polymerised DNA precipitates in the presence of 1N HCl and makes the medium opaque. If the organisms produce DNase enzymes, in sufficient quantity to hydrolyse the DNA, then clear zones are seen around the colonies.

Principle and Interpretation:

Tryptose is a source of nitrogen, vitamins, amino acids and other essential growth nutrients. Deoxyribonucleic acid (DNA) can be hydrolysed by microorganisms producing DNase. If the medium is then flooded with 1 N HCl not hydrolysed DNA precipitates (turbidity) and around DNase-positive colonies clear zones can be observed. Sodium chloride maintains the osmotic balance of the medium and Agar is the solidifying agent.

6. Mueller-Hinton agar, Oxoid, CM0337 Basingstoke, Hampshire, England Procedures to conduct antibiotic susceptibility test Source: Quinn *et al*. (1999).

Preparation of the inoculum

Inoculation of 6 to 7 distinct colony in to 5ml of saline was made first. Then the turbidity is compared with 0.5 MacFarland standard. This standard was prepared by adding 0.5 ml of 1 %(11.75g/litre) Bacl2.2H20to 99.5ml of 1 % (0.36N) H2SO4.

Inoculation to Mueller-Hinton agar, Oxoid, CM0337 Basingstoke, Hampshire, England

For slow growing bacteria, streptococci and corynebacterium species, 7% whole blood added Mueller-Hinton Agar was used. A sterile cotton swab on a wooden applicator stick was used to transfer the diluted bacterial suspension to a plate; excess fluid was squeezed out by rotating the swab against the sides of the tube. The plate was seeded uniformly by rubbing the swab against the entire agar surface in three different planes.

Disc application

Within 15 minutes (time used to dry the inoculum) after the plates were inoculated, antibiotic impregnated discs were applied to the surface of the inoculated plates by hand using a sterile forceps. All discs gently pressed down on to the agar with forceps to ensure complete contact with the agar surface. The discs were no closer than 1.5 cm to the edge of the plate and they were rest 3 cm apart from each other.

Incubation

The plates were incubated inverted aerobically for 24 hours at 37^0C

Interpretation

Inhibition zone was measured in millimeters using a transparent ruler on the under surface of the Petri dish. For measuring purpose, the end was taken as complete inhibition of growth as determined by naked eye. The result was interpreted according to the Table presented below taken from Quinn *et al*. (1999).

Appendix 5. Gram's stain (Atlas *et al.,* 1995).

The specimen was applied to a clean slide, then the specimen was fixed by heat. Crystal violet was applied (for 1 minute) and excess stain was washed, then Gram's iodine was applied (for 1 min.) and the excess was washed with distilled water. Alcohol decolorizing agent was applied and the excess was washed, after that safranin was applied (for 30 second) and the excess was washed, and finally the slide was dried for examination under the microscope.

Appendix 6. Distinguishing features of Coagulase positive staphylococci species.

Spps	Colony color	Hemolysis on sheep blood agar	Coagulase production	
			Tube	Slide
S.aureus	Golden yellow	+	+	+
S.intermidius	white	+	+	V
s.hyicus	white	-	V	-

V=Variable

Source: Quinn *et al.,* 2011

Hemolysis

Some bacteria produce hemolysins, exotoxins that cause red blood cells (RBC's) to burst open (hemolyse). When these bacteria are cultured on blood agar, this hemolysis is visible as an area of clearing around the colony (zone of hemolysis). If the organism produces enzymes that completely lyse the RBC's, this is termed beta hemolysis. Partial destruction of the RBC's produces a greenish color to the zone of hemolysis and is termed

Appendix 6. CAMP test result

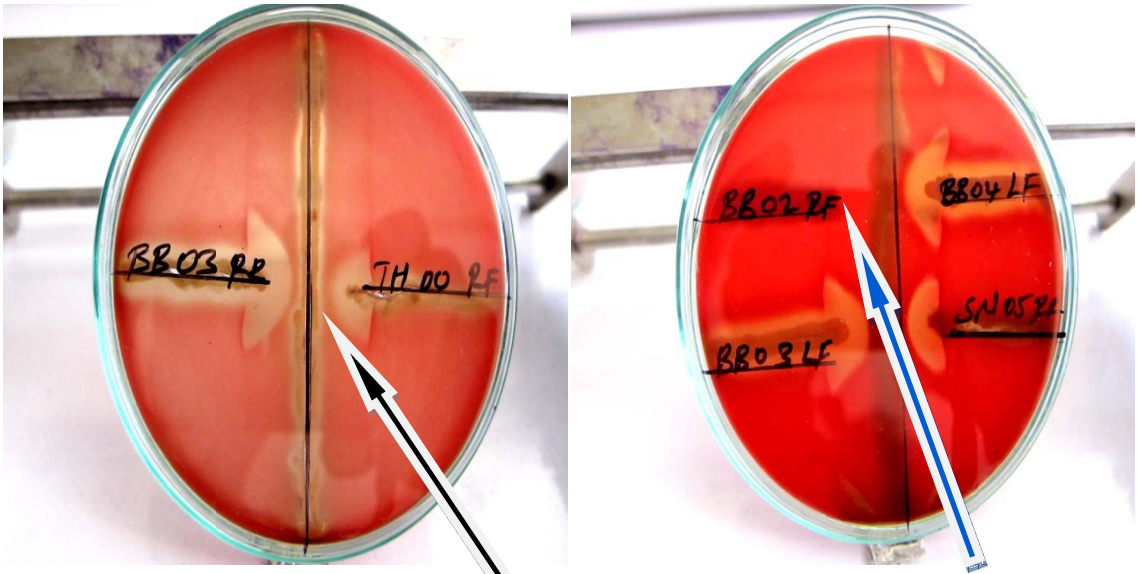

Figure 3. CAMP +Ve (S.agalactae) CAMP -Ve (S.Dysagalactae)

Appendix 8. Antibiotic susceptibility test result

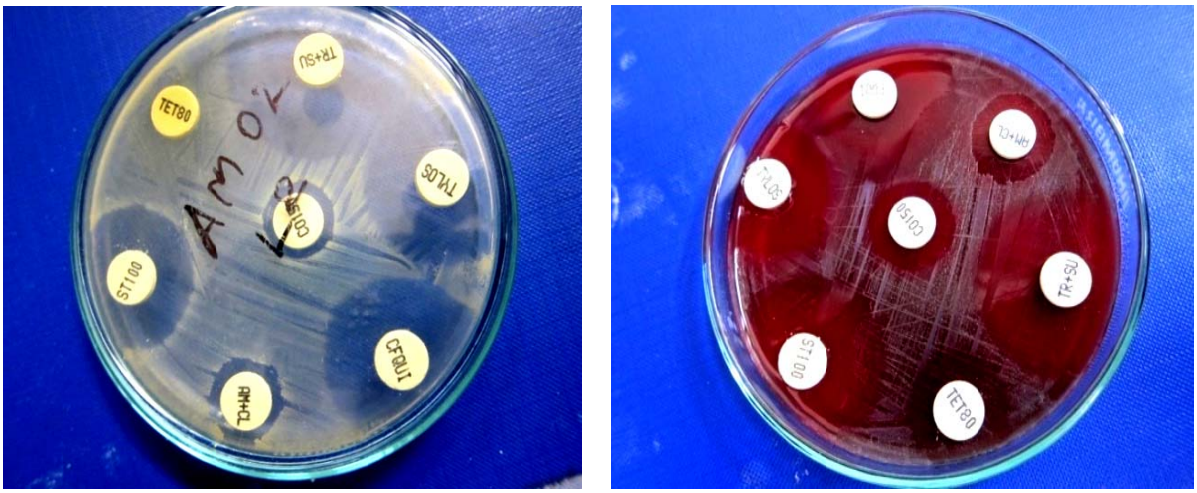

Figure 4. Antibiotic susceptibility test for *S.aureus and* S.agalactae

Appendix 8. Breakpoints used for *Staphylococcus* species from animals.

Antimicrobials	*Staphylococcus spps*		
	Breakingpoints zone[1], mm		
	R	**I**	S
Tetracycline (80 µg)	**≤ 23**	**24-27**	**≥ 28**
Chloramphenicol (60 µg)	**≤ 23**	**24-27**	**≥ 28**
Ampicillin (33 µg)			
Penicillin G (5 µg)	**≤ 27**	**-**	**≥28**
Cefalexin (30 µg)	**≤ 18**	**19-25**	**≥ 26**
Sulfonamides + trimethoprim	**≤ 23**	**24-27**	**≥ 28**
(240+ 5.2 µg)			
Lincomycin (19 µg)	**≤ 23**	**24-27**	**≥ 28**
Neomycin (120 µg)	**≤ 19**	**20-22**	**≥ 23**
Streptomycin (100 µg)	**≤ 23**	**24-27**	**≥ 28**
Enrofloxacin (10 µg)	**≤ 20**	**21-23**	**≥ 24**

Cefquinome (30 µg)	**≤ 19**	**20-22**	**≥ 23**
Polymyxins (150 µg)	**≤ 19**	**-**	**≥ 20**
Tylosin (150µg)	**≤22**	**23-25**	**≥26**
Oxacillin1 µg	**≤ 13**	**14-15**	**≥ 16**
Ampicillin33 µg	**≤ 20**	**21-27**	**≥ 28**

Source: Rosco 1994: Veterinary practice, Semi-confluent growth, ICS standard for fast growing bacteria, Mueller-Hinton agar.