# ISOLATION AND ANTIMICROBIAL RESISTANT PROFILE OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM DAIRYCOWS IN AND AROUND ASOSSA TOWN, BENISHANGUL GUMUZ REGIONAL STATE, WESTERN ETHIOPIA

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Abstract: A cross - sectional study was conducted from January 2024 to July 2024 in Dairy cattle in and around Asossa town in order to estimate the prevalence of mastitis, isolate and identify *S.aurues* from mastitic lactating cows, assess its antimicrobial resistance pattern and identify risk factors associated with mastitis. A total of 369 Dairy cows milk samples were collected with purposive sampling techniques. The overall prevalence of mastitis at cow level was 39.56 % with 14.90 % and 24.66 % of clinical and subclinical mastitis prevalence, respectively. A total of 1476 quarters were examined to detect clinical and subclinical mastitis by physical examinations of udder, milk and by California Mastitis Screening Test. From a total of 1476 quarters examined, 13.41 % (198/1476) and 23.37% (345/1476) of quarters were affected by clinical and sub clinical mastitis, respectively. In this study, the subclinical mastitis was higher than clinical mastitis. For all except Age and parity, intrinsic and extrinsic risk factors showed significant value for the prevalence of mastitis in the study area ($P<0.05$). From 146 mastitis infected lactating cows, 543 milk samples were cultured and 22.22% *S.aureus* were isolated. The present result showed a significant association of resistance pattern with *S.aureus* isolates, particularly to amoxicillin (84.61%), penicillin G (78.84%), Cefoxitin (76.92%), Tetracycline (69.23%), Streptomycin (61.53%), Gentamycin (53.84%), and Vancomycin (61.53%). In this study, 76.92 % *S.aureus* isolates were resistant for Cefoxitin. There were also observed multidrug resistance, mainly to Penicillin G, Streptomycin and Tetracycline. The present study revealed higher prevalence of mastitis and occurrence of resistance *S.aureus*, which are dependent on multiple associated risk factors. *S.aureus* to various antimicrobials indicated that, there is existence of resistance for frequently isolated mastitis bacteria to commonly used antimicrobial agents in the study area. Hence, regular resistance follow-up, using antimicrobials sensitivity tests helps to select effective antibiotics and to reduce the problems of drug resistance developments towards commonly used antimicrobials.

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## INTRODUCTION

Ethiopia has the largest cattle population in Africa with an estimated population of 52.13 million (CSA, 2007) and contributes 40% to the annual agricultural output, and 15% total gross domestic product. Cattle produce a total of 1.5 million tonnes of milk and 0.331 million tonnes of meat annually (FAO, 2005). Cows represent the biggest portion of cattle population of the country, around 42% of the total cattle heads are milking cows (CSA, 2008). However, milk production often does not satisfy the country's requirements due to a multitude of factors. Mastitis is among the various factors contributing to reduced milk production (Biffa *et al*., 2005).

Staphylococcosis, an infectious bacterial zoonosis of global significance, is caused by *S. aureus*, which is gram-positive, non-capsulated, non-motile, catalase positive, non-sporulated organism, grape-like clusters, 0.5-1.5 micrometer in diameter (Harris *et al*., 2002). Pathogenic *Staphylococci* are commonly identified by their ability to produce coagulase, and thus clot blood. This distinguishes the coagulase positive strains, *S. aureus, S. intermedius* and *S. hyicus* from the other Staphylococcal species such as *S. epidermidis* that are coagulase-negative (Harris *et al*., 2002). *S. aureus* is both commensal and pathogen. It is found as a commensal associated with skin, skin glands and mucous membranes. *S. aureus* affects skin, soft tissues, bloodstream and lower respiratory tract. It also causes severe deep-seated infections like endocarditis and osteolmyelitis (Schito, 2006). *S. aureus* also causes severe animal diseases, such as suppurative disease, arthritis and urinary tract infections (Lowy, 1998).

*S. aureus* is present in a variety of locations in the dairy farms, in many occasions it was isolated from swabs taken from the cows head, skin swabs, legs and nasal mucosa (Zadoks *et al*., 2000). Furthermore *S. aureus* was found on the milkers' hands as well as on the nasal mucous membrane of the humans working at the dairy farms, in bedding

and the drinkers (Benić *et al*., 2012). How ever an infected udder quarter remains the main reservoir of the bacteria, which transmitted mostly during the milking time. Recent researches show that many biotypes and genotypes exist on the dairy farms (Zadoks *et al*., 2002; Smith *et al*., 2005).

*S. aureus* plays its most significant animal pathogenic role as cause of intramammary infections in cattle and small ruminants leading to considerable economic losses in dairy farms. The pathogen is frequent causative agent of clinical or subclinical mastitis in cattle (Asperger and Zangerl, 2003). Presence of *S. aureus* on the skin and mucosae of food producing animals, such as ruminants, and the frequent association of the pathogen with mastitis, often leads to contamination of milk (Jablonski and Bohach, 1997). Contamination of milk can also occur from environmental sources during handling and processing (Peles *et al*., 2007). Milk is a good substrate for *S. aureus* growth and dairy products are common sources of staphylococcal food-poisoning (Morandi *et al*., 2007).

Enterotoxin-producing *S.aureus* plays an important role as causative organism of food intoxications. In many countries, *S. aureus* is considered to be the second or third most common pathogen causing outbreaks of food poisoning only outnumbered by Salmonella species, and in competition with Clostridium perfringen (Aycicnek *et al*., 2001).

Although a variety of antibiotics can be used against this organism, *S. aureus* mastitis has been found to respond poorly to antibiotic treatment (Barkema *et al*., 2006). The increased resistance *of S. aureus* isolates to several antimicrobial agents has been reported (Gentilini *et al*., 2000). The determination of antimicrobial susceptibility of clinical isolates is required not only for therapy but also for monitoring the spread of resistant strains throughout the populations. ß-lactam antibiotics are the most frequently used in intramammary infusion therapy. Bacterial resistance mechanisms to this class of antibiotics include production of ß-lactamase and low-affinity penicillin-binding protein 2a (PBP 2a) determined by the presence of the chromosomal gene mecA. The latter, designated for methicillin resistance, precludes therapy with any of the currently available ß-lactam antibiotics, and may predict resistance to several classes of antibiotics (Moon *et al*., 2007).

The usage of antibiotics correlates with the emergence and maintenance of antibiotic resistant traits within pathogenic strains (Shitandi and Sternesjo, 2004). These traits are coded for by particular genes that may be carried on the bacterial chromosome, plasmids, and transposons or on gene cassettes that are incorporated into integrons (Rychlik, 2006), thus are easily transferred among isolates. Multiple antibiotic resistant *S. aureus* strains have been isolated from milk obtained from cattle samples in many parts of the world (Pesavento *et al*., 2007).

From a number of epidemiological studies of Staphylococcal mastitis conducted, only few of them were done on economic and zoonotic significance *S. aureus* and MRSA strains from milk samples in Ethiopia. In Ethiopia there are few studies in Hawasa by (*Daka et al*.,2012), in Adama by (Abera *et al*., 2013), in and around Addis Ababa (Abebe *et al*., 2013). And hence, knowledge of zoonotic and economic impact of *S. aureus* and treatment failure in developing countries is necessary to make decisions and prerequisite for establishing control strategies.

Therefore, the objectives of the current study are:

- To determine the prevalence of bovine mastitic *S. aureus*
- To Isolate and characterizae *S. aureus* from mastitic lactating cows
- Assessment of the risk factors associated with *Staphylococcus* infections
- To determine the antimicrobial ressitance pattern of *S. aureus* species

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted in and around Asossa Town. Asossa is the capital city of the Benishangul-Gumuz Regional State and composed of 74 administrative peasant associations, which is located at 8°30'and 40°27' N latitude and 34°21' and 39°1' E longitude 687 kms Northwest of Addis Ababa (CSA, 2015). The altitude of Asossa ranges from 580 to over 1544 meter above sea level. The area is characterized by low land plane agro- ecology according to National Meteorological Service Agency (NMSA, 2014) with average annual rainfall of 1316 mm with uni-modal type of rainfall that occurs between April and October. Its mean annual temperature ranges between 16.75°C and 27.9°C. Asossa zone has 35.6% of the livestock population of the region constituting 61, 234 cattle, 191, 83 goats, 19,729 sheep, 25,137 donkeys, 439,969 poultry and 73,495 beehives (CSA, 2015), and the Asossa District has 16,990 cattle, 30,728 shoat, 57,089 poultry and 5,240 donkey (Bureau of agriculture, 2020)

Figure 1: Administrative map of Assosa town (ATAO, 2021).

**2.2. Study Design**

A cross - sectional type of study was conducted from January 2024 to July 2024 for isolation of *S. aureus* from CMT positive cows in the study area.

**2.3. Study Population**

The study population were Dairy cows owned by randomly selected peasant associations of small household farmers in and around Assosa District.

***2*.4 Sample size determination**

The total sample size for raw milk collection, isolation and enumeration of *S. aureus* was assigned according to statistical formula of Thrustfield (2005). A 5% absolute precision at 95% confidence interval was used during determining the sample size. Melaku T *et al*., (2021) who reported 40% of cow level mastitis due to S.aureus in and around asossa town. So, the expected prevalence was 40% according to previous study (2021). Therefore, the total sample size for the study were calculated as follows:

$$n = \frac{(1.96)^2 x\ P\ (1-P)}{d^2}$$

Where: n = the total sample size, P = expected prevalence (40%)
d = desired absolute precision (5%)
(0.05) at 95% CI

n = (1.96) x (1.96) x (0.4) x (1-0.4)/( (0.05) x (0.05) = 369 so, 369 cows were sampled from small house hold farms in the study.

**2.5 Sampling method**

For Dairy cows, milk samples were collected by a simple randomization technique. Strict aseptic procedure was followed when collecting milk samples in order to prevent contamination with micro organisms present on the skin udder and teats, on the hands of samplers and on the barn environment. Teat ends was cleaned and disinfected with ethanol (70%) before sampling. Strict foremilk (first jets) was discharged to reduce the number of contamination of teat canal (Quinn *et al.,* 1999). Sterile universal bottle with tight fitting cups was used. The universal bottle will be labeled with permanent marker before sampling. To reduce contamination of teat ends during sample collection, the near teats was sampled first and then followed by the far ones (Quinn *et al*., 1999).

Milk samples was collected from each of clinically and sub clinically mastitic non-blind quarters of the selected lactating cows for bacterial isolation, according to the National Mastitis Council Guideline (2004). After milking out and discarding the first two drops, about 2ml of milk was tested on CMT paddle from each quarter and about 25ml of milk was aseptically collected from each mastitis positive quarter using sterile universal bottle. Finally, the milk samples was transported immediately in an ice box to Regional Veterinary Laboratory of Benishangul Gumuz, Asossa, for microbiological examination. If immediate inoculation is not convenient, samples was kept at 4°C until cultured for isolation.

**2.6. Study Methodology**

*2.6.1. Questionnaire survey*

Data on each sampled cow was collected using a properly designed questioner format for determining the associated risk factors. This includes milker status, enviromental contamination, age, body condition, parity, and stage of lactation, breed, previous history of mastitis treatment, barn floor type, milking hygiene, milking practice and other relevant information related to other managemental practices related to mastitis will be gathered. Udder and milk abnormality (injuries, swelling, milk clots and abnormal secretions, etc) were also recorded (Appendix 1). Drug usage practice in the study area will be also collected to evaluate its contribution to the emergence of antimicrobial resistance strains from the study area of lactating dairy cows.

*2.6.2 Clinical Inspection of the Udder*

Udders of the cows was examined by visual inspection and palpation for the presence of any abnormalities. In addition, milk from each quarter was withdrawn and checked for any change in color and consistency (Quinn *et al*., 2002).

*2.6.3 California Mastitis Test (CMT)*

The California mastitis test were conducted to diagnose the presence of sub clinical mastitis and it will be carried out according to standard procedures. A squirt of milk from each quarter of the udder were placed in each of four shallow cups in the CMT paddle and an equal amount of the reagent was added. A gentle circular motion was applied in a horizontal plane. Positive samples showed gel formation within a few seconds. The result was scored based on the gel formation and categorized as negative if there was no gel formation, or positive if there was gel formation ranging from +1 to +3 (Appendix 2). If at least one quarter was positive by the CMT then the cow was considered as positive (Quinn *et al*., 1994).

*2.6.4 Culturing procedures*

Isolation and identification of *S. aureus* was conducted at Asossa Regional Veterinary Laboratory, on arrival in the laboratory, aliquots (centrifuged milk sample) of 0.01 ml of milk was streaked on blood agar (Oxoid, UK) containing 7% sheep blood for isolation of *Staphylococci.* The incubation was done aerobically at 37 °C for 24-48 hrs. The presence of more than 3 colonies of a similar morph-type was accepted as positive bacteriological finding (Ebrahimi *et al*., 2010). Identification of the bacteria on primary culture was made on the basis of colony morphology, haemolytic characteristics, Gram stain reaction including shape and arrangements of the bacteria, Catalase test and Oxidase test. In addition, growth characteristics on Mannitol salt agar and purple agar and tube coagulase test was conducted for specifically identifies *Staphylococcus* species (Genta and Heluane, 2001).

*Gram's staining*

Gram staining is the common, important, and most used differential staining techniques in microbiology, which was introduced by Danish Bacteriologist Hans Christian Gram in 1884, that imparts different colors to different bacteria or bacterial structures. Usually it differentiates bacteria into two groups; gram positive and gram negative. Hence after the gram staining, the gram positive cells appear as purple and gram negative cells appear as pink (Quinn *et al.*,2002). The study of morphological features and staining characteristics help in the preliminary identification of the isolate. The Gram stained smears from typical colonies that showed Gram-positive cocci occurring in bunched, grape like irregular clusters which will be taken as presumptive Staphylococcus species *(*Quinn *et al., 2002).*

*Mannitol salt Agar (Mannitol fermentation)*

The colonies that was confirmed by gram's staining reaction, haemolysis on the blood agar, colony characterization, catalase positive and oxidase negative was selected and streaked on Mannitol salt agar plate and incubated at $37^0$C and examined after 24-48 h for growth. The presence of growth and change of PH in the medium (red to yellow) was regarded as presumptive identification of *S. aureus* or coagulase positive *S. aureus.*

Phenol red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by *S. aureus* causes yellow discolouration of the medium with in 24 hrs of incubation (Mahon and Manusekis, 1995).

*2.6.5 Biochemical tests*

Isolation and identification of *S. aureus* is done according to standard techniques (Quinn *et al.,* 2002; ISO 6888-2, 2003).

The final identification of the organism and species assignment can be done based on Gram staining, catalase test, carbohydrate dissimilation (manitol ad maltose) fermentation and coagulase test by using rabbit plasma (ISO 6888-2, 2003).

*Catalase Test*

Pure culture of the isolates to be tested for catalase was picked up by bacteriological loop from the agar plate and mixed with a drop of 3% hydrogen peroxide on a clean slide. When the organism was positive, bubbles of oxygen was liberated within a few seconds. Those positive cocci were considered as Staphylococci (Quinn *et al*., 2002; Wilkinson, 1997).

*Oxidase Test*

A piece of filter paper will be moistened in a petridish with 1 percent aqueous solution of tetramethyl –p-phenylenediamianedihydrochloride. The test colony will be streaked firmly across the filter paper with a glass rod. The disappearance of dark purple color along the streak on the filter paper was considered as Staphylococcus (Quinn *et al*., 2002)

*Coagulase Test*

Coagulase test is used to differentiate *S. aureus* (positive) which produce the enzyme coagulase, from *S.epidermidis* and *S.saprophyticus* (negative*)* which do not produce coagulase. i.e Coagulase Negative *Staphylococcus* (CONS) (Hebert *et al*., 1988). Coagulase is an enzyme-like protein and causes plasma to clot by converting fibrinogen to fibrin. *S. aureus* produces two types of coagulase, i.e. free coagulase and bound coagulase. Free coagulase is an extracellular enzyme that can be detected in tube coagulase test while bound coagulase is a cell wall associated protein A that can be detected in slide coagulase test (Quinn *et al*., 2002).

*Purple agar Base (1% maltose fermentation)*

Purple agar base (PAB) with the addition of 1 percent maltose was used to differentiate the pathogenic *Staphylococci*, particularly the coagulase-positive isolates. The suspected culture was inoculated on PAB media plate (Defico) with 1% of maltose and incubated at $37^0$C for 24-48 hours. The identification was based on the fact that *S. aureus* rapidly ferment maltose with in 24 hrs and the acid metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow. The rapid fermentation (24hrs) was considered as *S. aureus* isolates (Quinn *et al*., 2002).

### 2.6.6 Antibiotic susceptibility testing

Determining of the type antibiotic for invitro sensitivity test, retrospective data was compiled on the type of antibiotics used to treat mastitis and other infectious diseases in the region of the study area. In addition to, the selection of the types of antimicrobial agents was made based on clinical considerations including frequent use of the drug in the study area and availability.

The *S.aureus* isolates were tested for anti-microbial susceptibility by disc diffusion method (Quinn *et al*., 2002). The following antibiotics were used for testing: Cefoxitin (30µg), Vancomycin (30µg), Penicillin G(10u), Tetracycline (30µg), Streptomycin (10µg), Chloramphenicol (30µg), Sulphamethoxazole trimethoprim (30µg) and Amoxacilin (30µg) Oxoid Company (Hampshire, England).

Colonies isolated from pure culture was transferred into a test tube of 5ml peptone and suspension was made and incubated at 37ºc for 8 hours. The turbidity of the suspension was adjusted comparing with that of 0.5 McFarland standards. Muller-Hinton Agar plate was prepared and a sterile cotton swab was dipped into the suspension and swabbed on the surfaces of Muller-Hinton Agar plate. Then, the antibiotic discs was placed on the agar plate using sterile forceps and pressed gently to ensure the complete contact with the agar surface. The plates were read after 24 hours of incubation at 35 0C under aerobic condition. The isolates was classified in accordance with the guideline of the National Committee for Clinical Laboratory Standards (CLSI, 2006) as susceptible, intermediate or resistance for each antibiotic tested according to the manufacturer's instructions by measuring the zone of inhibition around the antibiotic disc. Intermediate results were considered as resistant (Huber *et al*., 2011). Multiple antibiotic resistant (MAR) phenotypes were recorded for isolates showing resistance to three and more antibiotics (Rota *et al*., 1996).

### 2.7. Data Management and Statistical Analysis

Processing of data was done by computer software. Data was coded and entered to MS Excel spreadsheet and checked for accuracy. After validation, it was transferred and processed using computer software SPSS version 20 for analysis. Pearson's chi-square tests were used when appropriate to analyze the proportions of categorical data. Odd ratio and 95% CI was computed, the 95% confidence level was used, and results were considered significant at ($P < 0.05$).

## 3. RESULTS

### 3.1. Prevalence of mastitic Dairy Cows

In this cross- sectional study, out of the total lactating cows examined, 146(39.56 %) mastitis prevalence was found to be affected with clinical and subclinical mastitis infection. During laboratory examination, 82/369(22.2%) of the *S.aureus species was isolated*. The relative proportional prevalence of *Staphylococus aureus* was 82/146(56.29%) and it was found to be statistically significant (P=0.000). The highest mastitic dairy cows distribution were observed in Amba-7(54.09%) while the lowest prevalence was seen in Amba-2(24.19%) as indicated in Table 1.

**Table 1**: Prevalence of mastitic Dairy cows using clinical examination and CMT in study sites

| Study Sites | No of animals examined | Positive | Prevalence% | CHI2 | p-value |
|---|---|---|---|---|---|
| Asossa 01 | 62 | 27 | 43.54 | 15.03 | 0.01 |
| Amba-2 | 62 | 15 | 24.19 | | |
| Amba-3 | 61 | 28 | 45.90 | | |
| Amba-7 | 61 | 33 | 54.09 | | |
| Amba-14 | 60 | 22 | 36.66 | | |
| Amba-15 | 63 | 21 | 33.33 | | |
| Total | **369** | **146** | **39.56** | | |

1,476 quarters were examined from 369 cows, overall mastitis prevalence at different study site level were (n=369, 39.56%), under clinical examination and CMT screening. The prevalence of mastitis amongst study sites has significant difference ( $X^2$=15.03, P=0.01) (Table 1).

**Table 2:** Prevalence of mastitis at breed level in cross breed and local zebu of Dairy cows

| Breed | No of animal examined | No of positive (%) | Chi2 | p-value |
|---|---|---|---|---|
| **Cross breed** | 116 | 58 (50%) | 7.70 | 0.006 |
| **Local zebu** | 253 | 88 (34.78%) | | |
| **Total** | 369 | 146 (39.56%) | | |

1476 quarters of milk samples were examined from 369 cows, local Zebu (n=253, 34.78%), and also (N=116, 50%) cross breeds, under clinical examination and CMT screening. The prevalence of mastitis between crossbreed and local zebu breeds has significant difference (P=0.006) as shown in Table 2.

**Table 3: Prevalence of Subclinical and clinical mastitis at breed level**

| Form of mastitis | Breed | No of animal examined | No of positive (%) | Chi2 | p- value |
|---|---|---|---|---|---|
| **Sub clinical** | Cross | 68 | 39 (57.35%) | 289.38 | 0.000 |
| | Local | 134 | 52(38.80) | | |
| **Total** | | 202 | 91 (45.04%) | | |
| **Clinical** | Cross | 48 | 27(56.25%) | | |
| | Local | 119 | 28 (23.53%) | | |
| **Total** | | 167 | 55 (32.93%) | | |

From subclinical mastitis examined, 57.35% and 38.80% of mastitis were observed in cross and local breeds respectively, Whereas, from clinical mastitis, 56.25% and 23.53% of mastitis prevalence were recorded in cross and zebu breeds respectively. In this study, mastitis prevalence was higher in cross breeds than zebu breeds, which has significant association between them ($p<0.05$) as indicated in Table 3).

*3.1.1. Prevalence of mastitis at cow level*

The overall prevalence of mastitis at cow level as determined by CMT and clinical examination was 146 (39.56%) from a total population of 369 cows; 55(32.93%) were clinical where as 91 (45.04%) were subclinical mastitis and

223(60.43%) was healthy cows. So, the relative prevalence of each mastitis type in cows was 37.67% and 62.32% clinical and sub clinical mastitis respectively (figure 2).

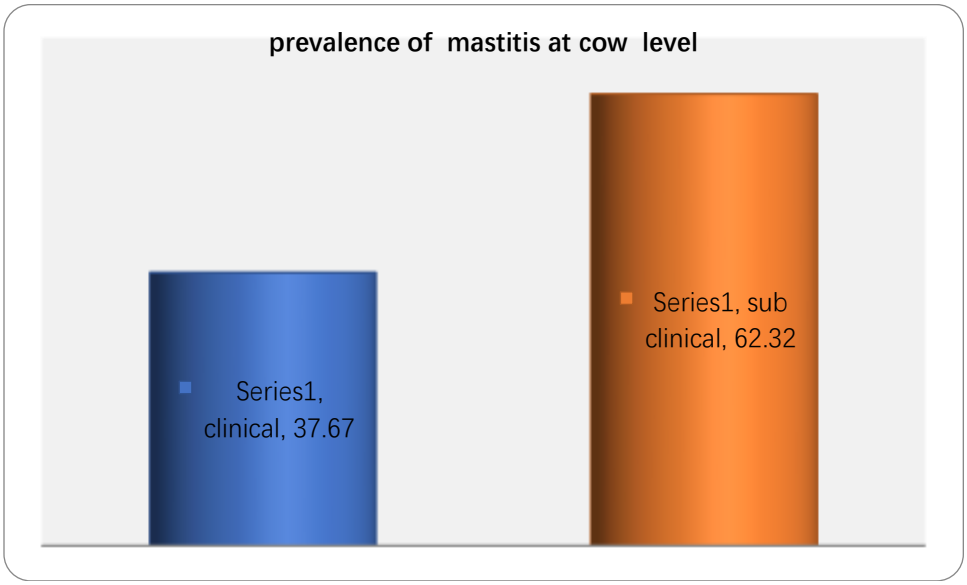


**Figure *2:*** Prevalence of different types of mastitis (n=146)

*3.1.2. Prevalence of mastitis at Quarter level*

Out of 1476 quarters examined using CMT and clinical examination methods, a total quarter of 543 (36.78%) were found affected by mastitis, 198(13.41%) and 345 (23.37%) were clinical and subclinical mastitis respectively. The proportational prevalence of each mastitis type was 198/543(36.46%) and 345/543 (63.53%) clinical and subclinical mastitis respectively. The relative quarter level prevalence of clinical and subclinical mastitis was 101 (18.60%), 163 (30.02%), 142 (26.15%), and 137 (25.23%) left front, left hind, right front and right hind respectively. And also 44.75% (243/543) front positive quarters and 55.24% (300/543) hind positive quarters of mastitis occurrence were indicated (Table 4).

**Table 4:** Prevalence of clinical and subclinical mastitis at quarter level by CMT and clinical examination

| **Form of mastitis** | **Quarter level** | | | | **Total** |
|---|---|---|---|---|---|
| | FL | HL | FR | HR | |
| **Clinical** | 31 | 61 | 50 | 56 | 198 |
| **Sub clinical** | 70 | 102 | 92 | 81 | 345 |
| **Total** | 101 | 163 | 142 | 137 | 543 |

**N=543**

**Key**: LF = left front, LH= Left hind, RF =Right front, RH = right hind

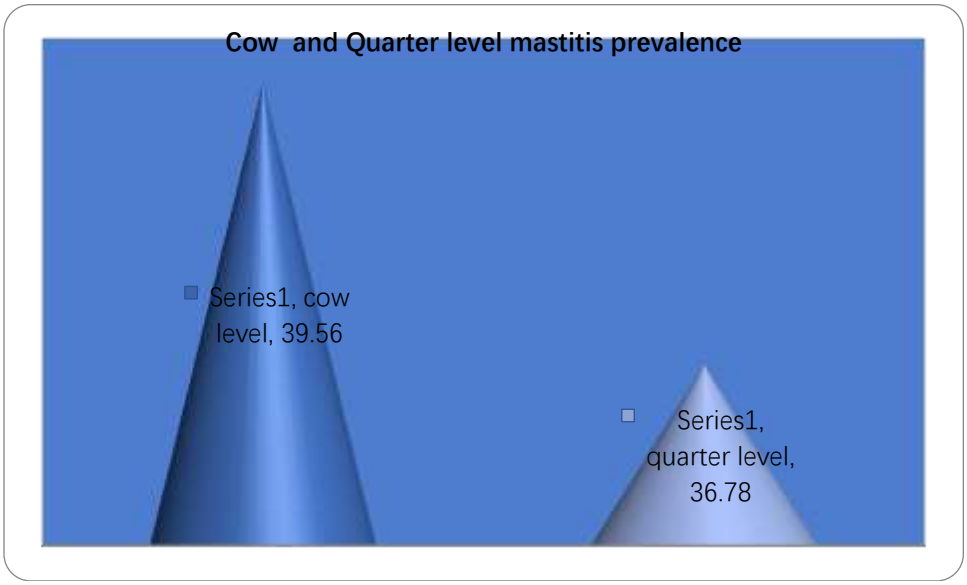


**Figure 2 Cow and Quarter level prevalence**

*3.1.3. Prevalence of S. aureus due to sub-clinical and clinical mastitis*

The overall cow level *Staphylococcus aureus due* mastitis was 82/369 (22.22%) and the contribution of *S.aureus* to clinical and subclinical mastitis were 23 (6.23%) and 59(15.98%) respectively (Figure 3).

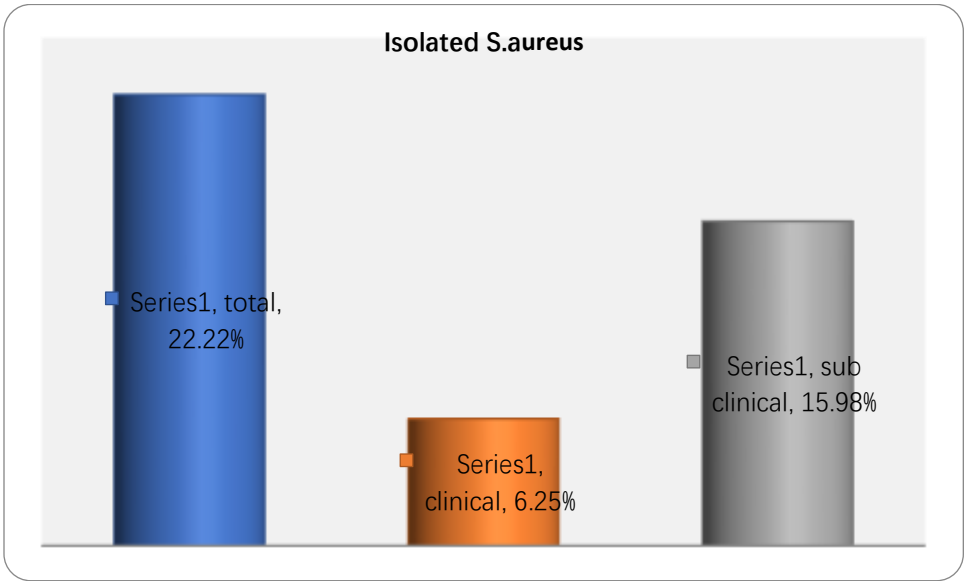


**Figure 3***:* Isolated *S. aureus*

### 3.2. Risk Factors Associated with mastitis Prevalence

Prevalence of mastitis related to the specific risk factors were determined as the proportion of affected cows out of the total examined. As indicated in (Table 8), the questionnaire survey and observation data result shows previous mastitis history and treatment history, milking hygiene, floor type, breed, lactation stage, and pregnancy status are amongst the potential risk factors, which are associated with mastitis disease in dairy cows farmstead. Accordingly, mastitis prevalence showed significant variation among different breed groups (p = 0.006), lactation stage (p=0.059), and pregnancy status

(p=0.006), previous mastitis history (p=0.000) and treatment history (p=0.000), milking hygiene (p=0.008), floor type (p=0.02). However, age and parity have no significant difference with mastitis (p>0.05) as shown in Table5.

**Table 5**: Result of multivariate logistic regression of attribute risk factors with mastitis

| Factor | Categories | Total no examined | No (%) positives | $X^2$ | p-value |
|---|---|---|---|---|---|
| Age(years) | ≥3- 5 (y-ad) | 128 | 51 (39.84%) | 1.40 | 0.49 |
| | >6 - ≥9 (adult) | 224 | 86 (38.39%) | | |
| | > 9 (old) | 17 | 9 (52.94%) | | |
| Breed | Cross | 116 | 58(50%) | 7.70 | 0.006 |
| | Zebu | 253 | 88(34.78%) | | |
| Parity | 1-2 | 198 | 76(38.38%) | 2.36 | 0.306 |
| | 3-4 | 118 | 44(37.28 %) | | |
| | ≥5 | 53 | 26(49.05%) | | |
| Lactation Stage (m) | Early (≤3) | 122 | 58(47.54%) | 7.45 | 0.059 |
| | Mid (4-6) | 131 | 41(31.29%) | | |
| | Late (7-9) | 78 | 30(38.46%) | | |
| | Dry (>9) | 38 | 17(44.73%) | | |
| Pregnancy Status | Pregnant | 100 | 28(28%) | 7.67 | 0.006 |
| | Non- Pregnant | 269 | 118(43.86%) | | |
| Previous mastitis History | Infected | 137 | 131(95.62%) | 286.33 | 0.000 |
| | Non- infected | 232 | 15(6.46%) | | |
| Floor type | Concrete | 247 | 87(35.22%) | 5.89 | 0.02 |
| | Muddy (soil) | 122 | 59(48.36%) | | |
| Milking hygiene | Good | 254 | 89(35.03%) | 6.98 | 0.008 |
| | Poor | 115 | 57(49.56%) | | |
| Prevoius mastitis Rx history | Yes | 93 | 89(95.69%) | 163.84 | 0.000 |
| | No | 276 | 57(20.65%) | | |

### 3.3. Occurrence of S. aureus from mastitic Dairy Cows

From 146 mastitis cases, infected Dairy cows (clinical 55 cows, subclinical 91 cows of 543 milk samples) were cultured and 82 *Staphylococcus aureus* were isolated. The proportional isolates of *Staphylococcus aureus* in clinical and subclinical mastitis was 82/146 (56.16 %). *S. aureus* was isolated at a rate of 23/146 (6.25%) and 59/146(15.98%) from clinical and subclinical mastitis infections, respectively. Statistically significant association was observed on the occurrence of *Staphylococcal* mastitis (Table 6).

**Table 6:** Number and percentage of *S. aureus* isolated from clinical case and CMT positive subclinical cows (543) quarters

| Form of mastitis | No of examined | No of *S.aureus* isolated | Prevalence % |
|---|---|---|---|
| **Clinical** | 167(198) | 23 | 6.25 |
| **Sub clinical** | 202(345) | 59 | 15.98 |
| **Total** | 369(543) | 82 | 22.22 |

**Key:** $X^2$ =289.38, p-value =0.000

### 3.4. Antimicrobial Susceptibility Test

From a total of 82 isolates of *S. aureus* obtained from the study, antimicrobial susceptibility tests were performed on 52 isolates. Due to the relatively small size, no separate analysis was undertaken for clinical and sub clinical isolates of *S.aureus* and were tested for antimicrobial sensitivity for 10 different types of antibiotics. The present study has demonstrated the existence of the levels of resistance of *S.aureus* to commonly used antimicrobial agents in the study area. 76.92 % of the *S. aureus* was found to be resistance to Cefoxitin. The resistance profile of Amoxicillin, Penicillin G, Tetracycline, Streptomycin, Vancomycin, Gentamycin, were 84.61, 78.84%, 69.23%, 61.53%, 61.53% and 53.84%, respectively (Table-9). In this study, *S. aureus* were found to be highly susceptible to Chloramphenicol (65.38%), Cloxacillin (63.46%), Trimethoprim-sulfamethoxazole (65.38%) and followed by gentamycin (40.38%) and streptomycin (38.46%). However, these isolates were highly resistant to Amoxicillin (84.61%), penicillin G (78.84%) and Cefoxitin (76.92%) followed by Tetracyline (69.23%). The antimicrobial resistance profiles are shown in Table 7.

**Table 7:** Resistance and susceptible of *S. aureus* isolates to different antimicrobials (n = 52).

| Antimicrobial agents | Disc content (µg) | No. of isolates | Resistance | Intermediate | Susceptible |
|---|---|---|---|---|---|
| | | | No (%) | No (%) | No (%) |
| Cefoxitin | 30 | 52 | 40(76.92) | 0 | 12(23.07) |
| TTC | 30 | 52 | 36(69.23) | 4(7.69) | 12(23.07) |
| Cloxacillin | 5 | 52 | 12(23.07) | 7(13.46) | 33(63.46) |
| Gentamycin | 10 | 52 | 28(53.84) | 3(5.76) | 21(40.38) |
| Streptomycin | 10 | 52 | 32 (61.53) | 0 | 20(38.46) |
| Penicillin G | 10 | 52 | 41(78.84) | 0 | 11 (21.15) |
| Chloramphenicol | 30 | 52 | 11(21.15) | 7(13.46) | 34 (65.38) |
| SXT | 25 | 52 | 15 (28.84) | 3(5.76) | 34(65.38) |
| Amoxycillin | 10 | 52 | 44(84.61) | 1(1.92) | 7(13.46) |
| Vancomycin | 30 | 52 | 32 (61.53) | 4 (7.69) | 16 (30.76) |
| **Mean** | | | 291 (5.6) | 29 (0.56) | 200(3.84) |

**Key**: SXT- Trimethoprim-sulfamethoxazole, S- Susceptible, I- Intermediate, R- Resistant

## 4. DISCUSSION

### 4.1. Overall prevalence

In the present study, the overall prevalence of mastitic dairy cows were 39.56 % in cows and 36.78 % in quarter level. This result was in line with the earlier reports by Biniam (2014) in and around Wolaita Sodo, Abinet (2015) in and around Batu town, Kerro and Tareke (2003) in Southern Ethiopia, (40.9% v 21%), (42.59% v 19.9%), (40% v 19%) in cows and quarters respectively. This report is relatively similar with the assertion by Radostits *et al*. (2000) that, in most countries and irrespective of the cause, the prevalence of mastitis is about 50% in cows and 25% in quarters. Besides, this result was in line with the findings of Bitew *et al*. (2010) at Bahir Dar, and Mulugeta and Wassie (2013), around Wolaita Sodo, 28.8%, 29.5% in cows respectively.

However, this finding is lower when compared with the previous findings of Shimelis (2014) in Selale/Fitche area, Alemayehu (2015) in Bahir Dar and its surroundings, Mesfin (2015) in and around Kombolcha, (83.1% v 65.42%), (62.06% v 42.44%), (56% v 33.7%) in cows and quarters respectively. In addition, it dis agrees with the previous findings of Sori *et al*. (2005) in and around Sebeta, Lakew *et al*. (2009) in Asella, Abaineh (1997) in Fiche, Abera *et al*. (2013) in Adama, Zerihun (1996) in Addis Ababa, Mekibib *et al*. (2010) in Holeta, Nesru (1986) in Dire-Dawa, 52.78%, 64.4%, 65%, 66.6%, 68.1%, 71.0%, 85.6% in cows respectively. This variability in prevalence of mastitis between different reports could be attributed to differences in farms management practice or to differences in study methods agro-climatic condition. As mastitis is a complex disease involving interactions of various factors such as managemental and husbandry, environmental conditions, animal risk factors, and causative agents, its prevalence will vary (Radostitis *et al.*, 2007).

### *4.2.* Prevalence of Clinical and Subclinical mastitis / S. aureus

Clinical mastitis is only the 'tip of the iceberg'. Sub clinical mastitis is by far the more costly disease in the majority of herds, and is often defined as the presence of a micro organism in combination with an elevated somatic cell count (SCC) of the milk. In this study, sub clinical mastitis is more prevalent than clinical mastitis at cow and quarter level and the same per individual quarter levels. This also provides further support of other studies in different region of the country which have concluded that subclinical mastitis is more prevalent than clinical mastitis.

Abinet (2015), Mesfin (2015), Biniam (2014), Alemayehu (2015), Shimelis (2014), Abera *et al*. (2013), Biffa *et al*. (2005), Mekibib *et al. (*2010) and Duguma *et al*. (2013) who have reported as (36.11% v 6.48%), (46% v 10%), (36.2% v 4.66%), (58.52% v 3.54%), (57.8% v 25.3%), (36.7% v 10%), (23.0% v 11.9%), (48.6% v 22.4%) and (73.3% v 7.8%) subclinical and clinical mastitis, respectively. This is likely to be partly influenced by virulence of the circulating bacterial strains and the levels of immunity of the cows to these pathogens. In addition, most smallholder farmers are not well informed or don't know about subclinical mastitis and they were surprised during our field work when they saw CMT positive milk reaction while it appeared to them normal milk before the test was conducted.

The occurrence of clinical mastitis in the present study was 14.90% (55/369) and that of subclinical mastitis was 24.66% (91/369) at cow level whereas 198/1476 (13.41%) clinical and 345/1476 (23.37%) subclinical mastitis in quarters. The prevalence of clinical mastitis in cows is in line with reports made by Mesfin (2015), in and around kombolcha, Kerro and Tareke (2003) in Southern and Hundera *et al*. (2005) in central Ethiopia with a rate of 10%, 10% and 16.11% respectively; but comparably lower findings of clinical mastitis at cow level is made by Abinet (2015), Almaw (2004) and Bitew *et al*. (2010) who reported 6.48%, 3.9% and 4.8% respectively in Bahir Dar, in and around Batu town, and 3.9% by Abera *et al*.(2013) in Adama, Ethiopia.

In addition, this finding is in line with the findings of Biniam (2014) who reported, 8.94 % clinical and 18.1 % subclinical mastitis in quarters and 36.2 % subclinical mastitis in cows**,** but it dis agrees with 4.66% clinical mastitis in cows, in and around Wolaita Sodo. However, the present research was comparably disagrees with the earlier findings of Alemayehu (2015) who revealed, 3.54% and 58.52 % of clinical and subclinical mastitis in cows, and 1.93% and 40.5% of clinical and subclinical mastitis in quarters respectively, in Bahir Dar. Besides, this finding is also lower as compard to the findings of Shimelis (2014) who revealed, 57.8% and 25.3% subclinical and clinical mastitis in cows, and 58.1% and 7.3% sub clinical and clinical mastitis in quarters respectively, in the Selale / Fitche Area. This variation in prevalence between subclinical and clinical mastitis may be due to the fact that, the defense mechanism of the udder reduces the severity of the disease (Eriskine, 2001).

The prevalence of mastitis in quarters of subclinical isolate 345/1476 (23.37%) disagrees with the findings of Duguma *et al*. (2013) who found 75.3 % subclinical mastitis whilst 6.0% clinical mastitis in Holleta agricultural research centre inline with present findings, 13.4%. In most reports including the present study, clinical mastitis is far lower than subclinical mastitis. This could be attributed to little attention given to subclinical mastitis, as the infected animal shows no obvious symptoms and secrets apparently normal milk and farmers, especially small holders, are not well informed about invisible loss from subclinical mastitis. In Ethiopia, the subclinical forms of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Almaw *et al*., 2008).

In this study, the prevalence of subclinical mastitis in cross and local breeds at cow level were 57.35% and 38.8% respectively whereas prevalence of clinical mastitis in cross and local breeds were 56.25% and 23.53% respectively.

The prevalence of subclinical mastitis of cross breed in cows was inconsistent with the findings of Zerihun (1996) in Addis Ababa dairy farms and Machang and Muyungi (1998) in Tanzania who reported prevalence of 55.1% and 67% respectively. That is significantly associated with the occurrence of mastitis. However, this report is lower as compared to the findings of Kivaria *et al*. (2004) in smallholder dairy farms in Tanzania and Alemayehu (2015) in Bahir Dar who reported prevalence of 90.3% and 73.79% in cross breeds of cows respectively.

The occurrence of subclinical mastitis in local breed at cow level was comparable with Temesgen (1999) in Mekele and Alemayehu (2015) in Bahir Dar who reported as prevalence of 25% and 28.57% respectively. The prevalence of clinical mastitis in cross and local breeds were high as compared to the previous reports of Alemayhu (2015) who showed prevalence of 4.85% and 0.95% in cross and local breeds respectively. The current study as well as in other similar studies, overwhelming cases of mastitis were subclinical as compared to clinical mastitis in both breeds (Kassa *et al*., 1999; Hussein, 1999; Workineh *et al.,* 2002; Kerro and Tareke, 2003). In Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Hussein, 1999) while the high economic loss could come from subclinical mastitis.

The quarter level mastitis recorded in the present study (36.78%) was in line with the report of Nesru *et al*. (1999) in Addis Ababa who reported 37%. Besides this, the current findings were consistent with reports of Abera *et al*. (2013) in Adama, and Mekibib *et al.* (2010) in Holeta, who reported 42% and 44.8% respectively; however, this finding was high, as compared with reports of Biffa *et al*. (2005) and Almaw (2004) who reported 28.2% and 23.0% respectively, and Kerro and Tareke, (2003) who found 19 % in different area of Ethiopia. This variation might be due to the complex effect of mastitis in the management system of the farm, breeds of cattle and geographical location of the study area.

In the present findings, the prevalence of mastitis in hind quarters was higher than front quarters, that is 55.24% and 44.75% respectively. This finding was in line with the findings of Biniam (2014) in and around Wolaita sodo, Mesfin (2015) in and around Kombolcha, Abinet (2015) in and around Batu Town, who reported (7.32% v 13.66%), (11.7% v 22%), (7.87% v 12.03%) of front and hind quarters mastitis respectively. This is due to the fact that the hind quarters are highly predisposed for contamination with dirt. In addition to this, large amount of milk is produced from hind quarters and as a result the pressure on the teat canal forces the canals to be opened widely which allows entrance of microbes.

In the present study, the prevalence of *S.aureus* in subclinical mastitis was 59/146 (40.41%) significantly higher than clinical mastitis 23/146 (15.75%). This finding was in line with the findings of Mesfin (2015) in Kombolcha, Abinet (2015) in Batu, Alemayehu (2015) in Bahir Dar, Biniam (2014) in Wolaita Sodo, Shimelis (2014) in Selale /Fitche, (19.33% v 7.33%), (12.96% v 4.17%), (14.50% v 0.51%), (12.06% v 3.11%) , (28.1% v 23.4%) subclinical and clinical isolates of *S. aureus* respectively. This is due to *S. aureus* is adapted to survive in the udder and usually establishes chronic subclinical infection of long duration from which it is shaded through milk serving as sources of infection for other healthy cows and transmitted during the milking process (Radostitis *et al*., 1994).

**4.3. Staphylococcal isolates in bovine mastitis**

With regard to the bacteriological analysis of milk sample, the relative isolates of ***S.aureus*** were 82/146(56.16%). This finding is inconsistent with the earlier findings of (51.56%) by Shimelis (2014), in Selale /Fitche Area, around Sebeta (44.03%) by Sori *et al*. (2005), in Holleta agricultural research centre (43.3%) by Duguma *et al*. (2013), in Hawassa area (48.75%) by Daka *et al*. (2012), in Holeta town (47.1%) by Mekibib *et al. (*2010) and in Debre Ziet area (39.5%) by Addis *et al.* (2011). Similarly, this result was inline with the previous findings of Bedada and Hiko, (2011), Workineh *et al*. (2002) and kerro and Tareke, (2003) who have reported as 39.1%, 39.2% and 40.3% *S. aureus* isolates at Assela, Addis Ababa and Southern Ethiopia, respectively. It was also closely comparable with findings of Lakew *et al*. (2009) and Ndegwa *et al*. (2000) who reported 41.1% and 43.3% in dairy cows, respectively.

However, *S. aureus* isolate is high as compared to the prevoius findings of Mesfin (2015) in Kombolcha, Abinet (2015), in Batu, Abebe *et al*. (2013), in Addis Ababa, by Seedy *et al* .(2010) in Egypt, Biniam (2014) in Wolta Sodo, Alemayehu (2015) in Bahir Dar, Hussein *et al*. (1997), Bishi (1998) and Mekuria *et al*. (2013), 26.7%, 17.13%, 16.0%, 17.2%, 18.39%, 15.02%, 10 %, 9%, 16% respectively. The high prevalence of this organism may be associated with its frequent colonization of teats, its ability to exist intracellular and localize within micro abscesses in the udder and

hence resistant to antibiotic treatment (MacDonald, 1997). The Bacteria usually establish chronic, subclinical infections and are shed in the milk, which serves as a source of infection for other healthy cows during the milking process. The possible explanation for the variation might be that *S. aureus* is a contagious pathogen transmitted from one cow to another or individual by contact with animals during unhygienic milking procedures (Rowe, 1999). Therefore, the *S.aureus* occurrence at a considerable high percentage indicates the alarming situation for dairy farms.

**4.5. Effects of potential risk factors on the occurrence of mastitis /S. aureus**

The prevalence of mastitis in local zebu and cross breeds were significantly associated with the occurrence of mastitis ($p=0.006$). Comparable research works were reported by Almaw *et al*. (2009) in Gondar town and its surroundings, Sori *et al*. (2005) in and around Sebeta showed that breed significantly influenced the occurrence of mastitis.

In addition, this finding was closely similar with Bitew *et al*. (2010) who reported in Bahir Dar, between Cross and Fogera breed, Lakew *et al*. (2009) in cross and local Arsi breed and Biffa *et al*. (2005) found significant difference between local Zebu, Holstein-Frisian and Jersey breeds in Ethiopia, That was Holstein Fresian pure breeds were affected at a higher rate both by clinical (26.3%) and subclinical (30.1%) mastitis than local breeds. Increased milk yield from genetic selection may be accompanied in genetic susceptibility to mastitis (Schutz, 1994). Besides this, the low occurrence of mastitis in local breeds in addition to genetic factors could also be one indication for higher occurrence of mastitis prevalence in areas where exotic breeds and their hybrids well adapted. Therefore, the lower prevalence in local zebu breeds in this study could be associated with difference in genetically controlled physical barrier like streak canal sphincter muscles, keratin in the teat canal or shape of teat end where pointed teat ends are prone to lesion (Seykora and Mcdaniel, 1985). In addition to physical barriers, the difference in occurrence of mastitis in these breeds could arise from differences in cellular immunity (Erskine, 2001).

The observed higher occurrence of mastitis during early lactation as compared to mid and late lactation stages was significant ($p<0.05$). The finding of higher infection in cows in early lactation stage followed by late and medium lactation stages in the study concurs with previous reports of Mulugeta and Wassie, (2013); Biffa *et al*. (2005) and Tamirat, (2007). In cows most new infections occur during the early part of the dry period and in the first two months of lactation (Radostits *et al*., 2007). This may be due to an absence of dry period therapy and birth related influences. During a dry period, due to low bactericidal and bacteriostatic qualities of milk, the pathogens can easily penetrate into the teat canal and multiply (Aylate *et al*., 2013). Radostits *et al*. (2000) suggested that, the mammary gland is more susceptible to new infection during the early and late dry period, which may be due to the absence of udder washing and teat dipping, which in turn may have increased the presence of potential pathogens on the skin of the teat. Moreover, during a dry period due to the low bactericidal and bacteriostatic qualities of milk, the pathogens can easily penetrate into the teat canal and multiply; this can be carried over into the post parturient period and ultimately develop into mastitis.

In this study, floor system had a significant influence on the occurrence of mastitis. In agreement with Abera *et al*. (2013) in Adama town and Fekadu *et al*. (2005) in southern Ethiopia, Lakew *et al*. (2009) and Sori *et al*. (2005). The findings of a high prevalence of mastitis in farms with muddy(soil) floors (48.36%) when compared with concrete floor types (35.22%) shows the occurrence of mastitis is significantly associated with the housing (bedding) type or condition of the farm. This is due to association with poor sanitation and cows which were maintained in dirty and muddy common barns with bedding materials that favor the proliferation and transmission of mastitis pathogens. The main sources of infection are udder of infected cows transferred via milker's hand, towels and environment (Radostitis *et al*., 2007).

Occurrence of mastitis was significantly associated with milking hygienic practice. Cows at farms with poor milking hygiene standard are severely affected (49.56%) than those with good milking hygiene practices (35.03%) (Mulugeta and Wassie, 2013; Lakew *et al*., 2009; Sori *et al*., 2005). This might be due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths, which could be vectors of spread especially for contagious mastitis (Radostitis *et al*., 2007).

In this finding the prevalence of mastitis was not significantly influenced by age categories ($P>0.05$). Similar result was reported by Shimelis (2014) in Selale /Fitche, no significant effect ($p>0.05$). In this study, parity is not

significantly influenced on the occurrence of mastitis (p>0.05). Incontrast to this study, the increased occurrence of mastitis with parity was reported by Mekibib *et al*. (2010) in Holeta town and Haftu *et al*. (2012) in northern Ethiopia.

**4.6. Antimicrobial susceptibility pattern**

The observations made in the present study unequivocally proved that *S.aureus* showed resistance to all antimicrobials tested. This shows that the existence of resistance of *S. aureus* to almost all commonly used antimicrobials in dairy farms and human medicine. *S. aureus* has a tendency to rapidly acquire antibiotic resistance to different classes of antibiotics.

In this study, the *invitro* disc sensitivity test showed that only two drugs have shown less resistant, 0 to 25% of the total isolates tested. These drugs were Chloramphenicol (21.15%) and Cloxacillin (23.07%**)** and followd by Sulphamethoxazole -trimethoprim (28.84%). Similar results with the finding of Abera *et al.* (2013), Abebe *et al.* (2013) in Adama and Jaims *et al*. (2002) who reported, less résistance of chloramphenicol and sulphamethoxazole-trimethoprim. The reason why these antimicrobials were less resistant might be that they are not used in the study area in veterinary clinics or services and even not frequently used (infrequent use of therapeutics) perhaps in human medicine.

The present study showed that the resistance of *S. aureus* to Amoxicillin (84.61%), Penicillin G (78.84%), Cefoxitin (76.92%), Tetracycline (69.23%), Cloxacillin (23.07%), Streptomycin (61.53%), Sulphamethoxazole-trimethoprim (28.84%), Chloramphenicol (21.15%), Vancomycin (61.53%) and Gentamycin (53.84%) observed in milk samples. Comparable research works were reported in various parts of Ethiopia by Biniam T (2014) revealed resistance of *S.aureus* to Penicillin G (100%), Cefoxitin (71.8%), Tetracycline (69.2%), Streptomycin (66.7%), Vancomycin (56.4%), Sulphamethoxazole–trimethoprim (43.6%) and Chloramphenicol (35.9%) in and around Wolaita Sodo, southern, Ethiopia**.** Besides this, Alemayehu (2015) indicated resistance of *S.aureus* to Penicillin G (95.8%), Cefoxitin (75.7%), Tetracyline (72.2%), Streptomycin (73.1%) and Vancomycin (52.4%) from Bovine mastitic milk in Dairy farms of Bahir Dar.

In addition, this research is in accordance with the findings of Abebe *et al.* (2013) who reported resistant of *S.aureus* to penicillin G 96.7% and tetracycline 73.8% around Addis Ababa, and Abera *et al*. (2010) 94.4% resistance to penicillin G in Adama; in addition to this study has demonstrated the existence of alarming level of resistance of *S. aureus* to commonly used antimicrobials (penicillin G, streptomycin and tetracycline) in dairy farms. This results were in consistent with reports from earlier studies in the other countries (Edward *et al*., 2002; Gentilini *et al*., 2002 and Jakee *et al*., 2008) suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. Hence, penicillin and tetracycline are the only most commonly used antimicrobials for the treatment of other infections as well as mastitis in veterinary practice in Ethiopia, as the result, there was spread of drug resistance reported by many researchers which was in line with the recent findings.

The resistance of *S.aureus* isolates to beta-lactam antibiotic was evident. High percentage of *S.aureus* was resistant to the most frequent drugs. In agreement with the finding of by Derese *et al*. (2012), the study showed cefoxitin resistant isolates were obtained from the milk. All cefoxitin resistant *S. aureus* were also resistant to penicillin G. Out of the (76.92%) cefoxitin resistant *S. aureus* isolates, (84.61%) and (78.84%) were also resistant to amoxicillin and Penicillin G respectivelly. This is an indicator of MRSA (Daka *et al*., 2012). This is due to the fact that resistance of *S. aureus* to these drugs may be attributed to the production of β-lactamase, an enzyme that inactivates penicillin and closely related antimicrobials (Wubishet *et al*., 2012; Sharma *et al*., 2011; Green and Bradely, 2004).

In the present observation, frequent multidrug resistance pattern were exhibited for Penicillin G, Cefoxitin and tetracycline. Comparably, Alemayehu (2015) who reported as resistance for multidrugs, mainly to penicillin G, Cefoxitin and tetracycline. In addition, Shimelis (2014) who found that, 86.46 % of the isolates were resistant to different combinations of two or above tested antibiotics and the most frequent multidrug resistance pattern consisting of three drugs' is exhibited for, gentamicin, ceftazidime and streptomycin with a resistance of 9.46% of the isolates. Similar finding by Mekuria *et al*. (2013) reported MRSA isolate with resistant to more than two of non-β-lactam antimicrobials. This multi drug resistance occurred might be due to administration of multiple antibiotics for prophylaxis or infection, lack of drug sensitivity tests in the dairy farms, uncontrolled or discriminate use of antibiotics

in the farms and another possibility is that cattle are being treated with antibiotics for other conditions, thereby selecting for resistant populations of *S. aureus* (Shitandi and Sternesjo, 2004).

## 5. CONCULUSION AND RECOMMENDATIONS

Clinical and subclinical mastitis could be one of the major constraints to dairy production in extensive dairy farms. Different potential risk factors are associated with mastitis in the study area, amongst these, Breed, milking hygiene, floor type, lactation stage, previous mastitis & treatment history and pregnancy status of the animal were prominent. Mastitis caused by *S. aureus at* cow and quarter level were one of the major problems of dairy cows in milk production. It was found that the majority of the tested isolates were resistant to the various antimicrobial agents especially amoxicillin, penicillin G, Cefoxitin, and Tetracycline. It was also observed that large proportions of the isolates were susceptible to Cloxacillin, Sulphamethoxazole-trimethoprim and Chloramphenicol. In the present observation, *S.aureus* isolates showed multi drug resistance primarily to Penicillin G, Cefoxitin, and Tetracycline. The present study revealed higher prevalence of mastitis and occurrence of multidrug resistance *S. aureus* specifically which belongs to the MRSA which are dependent on multiple associated risk factors.

Based on the above conclusion the following points are forwarded:-

- Mastitis control strategy should be initiated and promoted in the study area;
- Awareness creation of dairy farms owners, dairy workers and veterinarians on the effect of MRSA should made
- Hygiene measures during milking procedure should be practiced that may reduce the transmission of the disease
- There should be regular antimicrobial sensitivity test to select effective and alteration of antibiotics to reduce the problems of drug resistance development towards commonly used antibiotics
- Impacts and dynamics of genetic antibiotic determinants should also be investigated using molecular methods.

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