Isolation, Identification and Antimicrobial Resistance Profile of Staphylococcus in Dairy Cows of Assosa town, Abrahamo, Ura and Bambasi Districts of Benishangul Gumuz Regional State, Western Ethiopia

Asmamaw Aki * and kebede Alga and Arega Muleta

Assosa, Regional Veterinary Diagnostic, Surveillance, Monitoring and Study Laboratory, P.O. Box 326, Assosa, Ethiopia; asmamawaki@gmail.com, phone: +251- 902330029

ABSTRAC: A cross - sectional study was conducted from November 2023 to July 2024 in Dairy cattle in Assosa town, Abrahamo, Ura and Bambasi districts in order to estimate the prevalence of mastitis, isolate and identify *S.aurues* from Dairy cows, assess its antimicrobial resistance pattern and identify risk factors associated with mastitis. A total of 385 Dairy cows milk samples were collected with random sampling techniques. In this study, out of the total lactating cows examined, 150(38.96%) mastitis prevalence was found to be affected with mastitic infection. During laboratory examination, 85/385(22.07%) of the *S.aureus* was isolated and 69/385(17.92%) of other coagulase negative *staphylococcus spp (CNS) were* identified. The relative proportional prevalence of *Staphyloccocus aureus* was 85/150(56.66%). They were found to be statistically significant (P<0.00). The highest mastitic dairy cows' distribution were observed in Abrahmo (51.9%) while the lowest prevalence was seen in Ura (29.33%). In this study, breed, age, parity, tick infestation, teat lesion were non- significant (P>0.05) while length of lactation, previous mastitis history, blind teat, previous mastitis treatment history, milk hygiene and floor type were significant (p<0.05). The present result showed a significant association of resistance pattern with *S.aureus* isolates, particularly to penicillin G (78.84%), Cefoxitin (76.92%), Tetracycline (69.23%), Streptomycin (61.53%) and Gentamycin (53.84%) were investigated. 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 so as to reduce the problem encountered**.**

[Asmamaw Aki and kebede Alga and Arega Muleta. Isolation, Identification and Antimicrobial Resistance Profile of Staphylococcus in Dairy Cows of Assosa town, Abrahamo, Ura and Bambasi Districts of Benishangul Gumuz Regional State, Western Ethiopia. *Life Sci J* 2024;21(12):14-30]. ISSN 1097-8135 (print); ISSN 2372-613X (online). http://www.lifesciencesite.com. 03. doi:10.7537/marslsj211224.03

Keywords: Assosa town, *Abrahamo, Bambasi, Dairycows, milk, Staphylococcus aureus, Ura.*

1. 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* from milk samples in Benishagul Gumuz region. In Benishagul Gumuz region, there are few studies in and Around Asossa town and also Bambasi District (Asmamaw A et al., 2017, 2018). 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.

So far, there was no study done on to assess the epidemiology of *Staphylococcus spp* in Assosa town, Abrahamo, Ura and Bambasi districts.

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 were conducted in Assosa town, Abrahamo, Ura and Bambasi districts. 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 Assosa District has 16,990 cattle, 30,728 shoat, 57,089 poultry and 5,240 donkey (Bureau of agriculture, 2016).

Bambasi district has 38 kebeles stretches over an area of 2210.16 square k.m with human population of 62693. The region is found in the north west of the country between latitude of 9 and 11^0N and longitude of 34 and 35^0E and its altitude range is 1500-1900 meter above sea level. Annual rain fall is between 1350-1400 mm with uni modal type of rain

fall that occurs between April and October. Annual temperature ranges between 21^0c - 35^0c. The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 36,735 Cattle, 10732 Goat, 3739 Sheep, 4467 Equines, 41438 Poultry and 23423 beehives (CSA, 2015).

Figure 1: Map of Benishangul Gumuz Regional state Source (Mulaw *et al*., 2011)

2.2. Study Design

A cross - sectional type of study was conducted from Nov 2023 to July 2024 for isolation of *S. aureus* from dairy cows in the study areas.

2.3. Study Population

The study population were Dairy cows owned by randomly selected peasant associations of small household farmers in study Districts.

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

The total sample size for raw milk collection, isolation and enumeration of *S. aureus* was assigned according to Thrustfield (2005) formula. 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

$n = (1.96) \times (1.96) \times (0.4) \times (1-0.4)/((0.05) \times (0.05) = \underline{369}$ So, 369 cows were sampled from small house hold farms in the study , however; it was increased to 385 to increase precision.

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.*, 2002). 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 sample 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 were 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. 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. Squirts 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 5-7% sheep blood for isolation of *Staphylococci.* The incubation was done aerobically at 37 °C for 24 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 base (1% maltose fermentation) and tube coagulase test was conducted for specifically identifies *Staphylococcus* species (Genta and Heluane, 2001).

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).

2.6.6 Antibiotic susceptibility test

In determining the type of 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 were 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), PenicillinG (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 5 ml peptone and suspension was made and incubated at 37^{o}c 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 ^{0}C 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 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 Stata version 12 for analysis. Pearson's chi-square tests was 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 as significant ($P < 0.05$).

3. RESULTS

3.1. Prevalence of mastitis

In this cross- sectional study, out of the total lactating cows examined, 150(38.96 %) mastitis prevalence was found to be affected with infection. During laboratory examination, 85/385(22.07%) of the *S.aureus was isolated and 69/385(17.92%) of other coagulase negative staphylococcus spp (CNS) were isolated*. The relative proportional prevalence of *Staphyloccocus aureus* was 85/150(56.66%). They were found to be statistically significant (P<0.00). The highest mastitic dairy cows distribution were observed in Abrahmo(51.9%) while the lowest prevalence was seen in Ura (29.33%) as indicated in Table 1.

Table 1: Prevalence of mastitic Dairy cows in study sites

Study Sites	No of animals examined	Positive	Prevalence (%)	Chi2	p-value
Asossa town	124	42	33.87	11.62	0.009
Abrahamo	104	54	51.9		
Bambasi	82	32	39.02		
Ura	75	22	29.33		
Total	385	150	38.96		

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 2), the questionnaire survey and observation data result shows previous mastitis history and treatement history, milking hygiene, floor type, and lactation stage, pregnancy status, and blind teat are amongst the potential risk factors, which are associated with mastitis disease in dairy cows farmstead. Accordingly, mastitis prevalence showed significant variation among different blind teat groups ($p = 0.000$), lactation length ($p=0.02$), and pregnancy status ($p=0.004$), previous mastitis history ($p=0.000$) and treatement history ($p=0.000$), milking hygiene ($p=0.005$), floor type ($p=0.01$). However, breed, age, tick infestation, teat lesion and parity have no significant difference with mastitis ($p>0.05$).

Table 2: Result of multivariate logistic regression of attribute risk factors with mastitis

Factor	Categories	Total no examined	No (%) positives	Chi2	p-value
Age(years)	≥3- 5 (y-ad)	137	53 (38.68%)	1.47	0.47
	>6 - ≥9 (adult)	231	88 (38.09%)		
	> 9 (old)	17	9 (52.94%)		
Breed	Cross	180	70(38.88%)	0.00	0.97
	Zebu	205	80(39.02%)		
Parity	1-2	209	80(38.27%)	2.83	0.24
	3-4	123	44(35.77%)		
	≥5	53	26(49.05%)		
Lactation Stage (m)	Early (≤3)	124	60(48.38%)	10.42	0.02
	Mid (4-6)	139	41(29.49%)		
	Late (7-9)	84	32(38.09%)		
	Dry (>9)	38	17(44.7%)		
Pregnancy Status	Pregnant	103	28(27.2%)	8.20	0.004
	Non- Pregnant	282	122(43.26%)		
Previous mastitis History	Infected	141	135(95.74%)	301.6	0.000
	Non- infected	244	15(6.14%)		
Floor type	Concrete	263	91(34.6%)	6.63	0.01
	Muddy (soil)	122	59(48.36%)		
Milking hygiene	Good	270	93(34.44%)	7.75	0.005
	Poor	115	57(49.56%)		
Prevoius mastitis Rx history	Yes	96	92(95.83%)	173.9	0.000
	No	289	58(20.06%)		
Blind teat	No	331	96(29.0%)	40.09	0.000
	Yes	54	54(100%)		
Tick infestation	No	331	134(40.48%)	2.29	0.13
	Yes	54	16(29.62%)		
Teat lesion	No	366	146(39.89%)	2.69	0.10
	Yes	19	4(21.05%)		

3.3. Antimicrobial Susceptibility Test

Antimicrobial susceptibility tests were performed on 22 staphylococcus isolates and were tested for antimicrobial sensitivity for 5 different types of antibiotics. The present study has demonstrated the existence of the levels of resistance of *S.aureus* to commonly used antimicrobial agents. 76.92 % of the *S. aureus* was found to be resistance to Cefoxitin. The resistance profile of Amoxicillin, Penicillin G, Tetracycline, and Gentamycin, were 84.61, 78.84%, 69.23%, and 53.84%, respectively (Table-3). In this study, *S. aureus* were found to be highly susceptible to Cloxacillin (63.46%) followed by Gentamycin (40.38%). However, these isolates were highly resistant to penicillin G (78.84%) and Cefoxitin (76.92%) followed by Tetracyline (69.23%). The antimicrobial resistance profiles are shown in Table 3.

Table 3: Resistance and susceptible of *S. aureus* isolates to different antimicrobials (n = 22).

Antimicrobial agents	Disc content (µg)	No. of Isolates	Resistance	Intermediate	Susceptible
			No (%)	No (%)	No (%)
Cefoxitin	30	22	17(77.27)	0	5(22.72)
TTC	30	22	15(68.2)	2(9.09)	5(22.72)
Cloxacillin	5	22	5(22.72)	3(13.63)	14(63.63)
Gentamycin	10	22	12(54.54)	1(4.54)	9(40.90)
Penicillin G	10	22	17(77.27)	0	5 (22.72)
Mean			66 (13.2)	6 (1.2)	39(7.8)

Key: S- Susceptible, I- Intermediate, R- Resistant

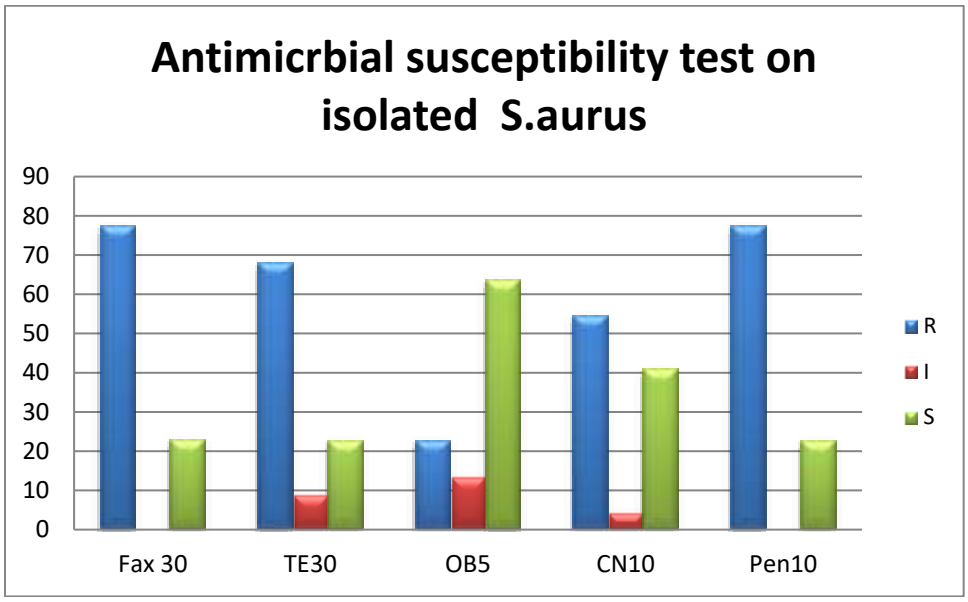

Figure 4: Antimicrobial drugs resistance and susceptibility profile of *S.aureus* isolated from milk
Key: TE30=Tetracycline, Fax30= Cefoxitin, CN10=Gentamycin, Pen10= Penicillin G, OB5= Cloxacillin.

4. DISCUSSION

In the present study, the overall prevalence of mastitic dairy cows were 38.96 % in cows. 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%), (42.59%), (40%) in cows 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).

With regard to the bacteriological analysis of milk sample, the relative isolates of ***S.aureus*** were 85/150(56.66%). 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.

The prevalence of mastitis in local zebu and cross breeds were in-significantly associated with the occurrence of mastitis ($p>0.05$). 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 ($p<0.02$). 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 ($p=01$). 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 (p=0.005). 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.

The present study showed that the resistance of *S. aureus* to Penicillin G (78.84%), Cefoxitin (76.92%), Tetracycline (69.23%), Cloxacillin (23.07%) 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%), and Tetracycline (69.2%) in and around Wolaita Sodo, southern, Ethiopia**.** Besides this, Alemayehu (2015) indicated resistance of *S.aureus* to Penicillin G (95.8%), Cefoxitin (75.7%), and Tetracyline (72.2%), 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, 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

Dairy cows mastitis could be one of the major constraints to dairy production in extensive dairy farms. Different potential risk factors were associated with mastitis in the study area, amongst these, length of lactation, blind teat, 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 was 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 penicillin G, Cefoxitin, and Tetracycline.

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

- Mastitis control strategy should be initiated and promoted in the study area;
- 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

6. REFERENCES

2. Abebe, M., Daniel A., Yimtubezinash W. and Genene T. (2013): Identification and Antimicrobial susceptibility of Staphylococcus aureus isolated from milk samples of Dairy cows and nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia. *Afr. J. Microbiol. Res.,* **7** (27):3501-3510.
3. Abera, B., Lemma D. and Iticha I. (2013): Study of Bovine Mastitis in Asella Government Dairy farm of Oromia Regional State, South Eastern Ethiopia.*Int. J. Curr.Res. Aca. Rev.,***1** (2):134-145.
4. Asperger, H. and Zangerl, P. (2003): Staphylococcus aureus. Ency. Dai. Sci., 2563–2569.
5. Asmamaw Aki et al., Isolation, Identification and Antimicrobial resistance profile of staphylococcus aureus and occurrence of methi cillin resistant S. aureus isolated from mastitic Dairy cows in and around Assosa town, Benishangul Gumuz Region, Ethiopia. Interna tional Journal of Animal Research, 2017; 1:5
6. Adams, M. R. and Moss, M. O. (2005): Food Microbiology. The Royal Society of Chemistry 2nd ed., Cambridge. UK. Pp. 253-259.
7. Andreasen, C. B. (2003): *Staphylococcosis*. In: Saif, Y. M., Barnes, H. J., Fadly, A. M., Glisson, J.R., McDougald, L. R. and Swayne, D. E. (eds.). Diseases of Poultry. 11th ed., A Blackwell Publishing Company. Iowa State Press. Pp. 798-804
8. Argaw, K. and Tolosa, T. (2007): Prevalence of sub clinical mastitis in small holder dairy farms in Selale, North Shewa 144 Zone, Central Ethiopia. *Inter. J. Vet. Med*. ISSN., **5**:1937-816
9. Arnold, M. and Bewley, J. (2011): *Staphylococcus aureus* mastitis. Cooperative extension service university of Kentucky, College of Agriculture, Lexington., ky 40546, UK.
10. Atanassova, V., Meind, A. and Ring, C.(2001): Prevalence of *Staphylococcus aureus* and Staphylococcal enterotoxins in raw pork and uncooked smoked ham-a comparison of classical culturing detection and RFLP-PCR. *Inter. J. of Food Microbiol.,* **68**:105-113.
11. Ateba,C.N., Mbewe, M., Moneoang, M.S., Bezuidenhout, C.C. (2010): Antibioticresistant *Staphylococcus aureus* isolated from milkin the Mafikeng Area, North West province, South Africa. *S .Afr.J Sci.,* **106**:1-6
12. Aycicek ,H., Cakiroglu, S. and Stevenson ,T. (2005): Incidence of *Staphylococcus aureus* in ready to eat meals from military cafeterias in Ankara, Turkey. *Food Control,* **16**: 531534.
13. Ayliffe, G. (1997): The progressive intercontinental spread of Methicillin-resistant Staphylococcus aureus. *Clin.Infec.Dis.,* **24**:74-79.
14. Bagcigil ,F.A., Moodley, A., Baptiste, K.E., Jensen ,V.F., Guardabassi, L. (2007): Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. *Vet. Microbiol.,* **121**:307–315.
15. Baker, J. S. (1984): Comparison of various methods for differentiation of staphylococci and micrococci. *J. Clin. Microbiol.,* **19**: 875–879.
16. Bannerman, T. L. (2004): *Staphylococcus, Micrococcus* and other catalase-positive cocci that grows aerobically In: Manual of Clinical Microbiology P. R. Murray, E. J. Baron, J. H. Jorgensen, `M. A. pfaller and R. H. Yolken eds. 8th edition, ASM Press Washington DC
17. Benntt, R.W .and Monday, S.R. (2003): *Staphylococcus aureus*. In Militos, M, D and Bier, J W ends. International Hand Book of Foodborne Pathogens. Marrcel Dekker, Inc. New York. Pp. 53-72.
18. Bens, C.C.P.M., Voss , A. and Klaassen, C.H.W. (2006): Presence of a novel DNA methylation enzyme in methicillin-resistant Staphylococcus aureus isolates associated with pig farming leads to un interpretable results in standard pulsedfield gel electrophoresis analysis. *J. Cl. Micr.,* **44**: 1875-1876.
19. Bhunia, A.K. (2008): Food borne Microbial Pathogens. Mechanisems and pathogenesis, Spring science and Business Media , LLC, New York., Pp. 125-134.
20. Biffa, D., Etana, D. and Fekadu, B. (2005): Prevalence and Risk Factors of Mastitis in Lactating Dairy Cows in Southern Ethiopia.

Intern. J. Appl. Res. Vet. Med., Hawassa College of Agriculture, Debub University, Hawassa, Ethiopia., **3**: (3), 189-198.

21. Breynestad, S. and Granums, P. E. (2002): Clostridium perfringens and food borne infection. *Inter. J. of Food Microbiol.*, **74**: 195-202
22. Brook, I. and Frazier, E.H (2001): Clinical features and aerobic and anaerobic microbiological characteristics of cellulites. *Arch. Surg.*, **130**:786-792.
23. Brouillette, E. and Malouin, F. (2005): The pathogenesis and control of *Staphylococcus aureus* induced mastitis, Study models in the mouse, *Micro. Infct.*, **7**:560-568
24. Brown ,D.F., Edwards, D.I., Hawkey, P.M., Morrison, D., Ridgway, G.L., Towner ,K.J. (2005): Guidelines for the Laboratory Diagnosis and Susceptibility Testing of Methicillin resistant *Staphylococcus aureus* (MRSA). *J. Antimicrob. Chemother*.,**56** (6):1000-18.
25. Bureau of Agriculture (2016): Livestock resource development and animal health Development Annual Report, Asosa, Ethiopia.
26. Burke, S.T. and Warren, E.R. (2014): New pharmacological treatments for Methicillin resistant *S. aureus* infections. *Expert opinion on pharmacotherapy,* **15**: 483-491.
27. Bustin, S.A. (2000): Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* **25:** 169-193.
28. Central Statistical Agency (CSA) (2008): Federal democratic republic of Ethiopia, central statistical agency. Agricultural sample survey, Volume 2, report on livestock and livestock characteristics.
29. Cheng, A.G., McAdow, M., Kim, H.K., Bae, T., Missiakas, D.M., Schneewin (2010): Contribution of coagulases towards Staphylococcus aureus disease and protective immunity. *PLoS. Pthog.*, **6**:1001-036
30. Cimolai, N. (2006): Community-acquired MRSA infection: An emerging trend. *BCMJ*., **48**: 116-120.
31. Clinical and Laboratory Standards Institute (CLIS) (2009): Methods for dilution Antimicrobial Susceptibility Testing for Bacteria that grew aerobically. Approved Standard M7-A10. Wayne, PA: Clinical and Laboratory Standards Institute.
32. Clinical and Laboratory Standards Institute (CLISI) (2008): CLSI document M23-A. 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute.Development of in vitro susceptibility testing criteria and quality control parameters; approved guideline.
33. Cohn,L.A. and Middleton, J.R.(2010): A Veterinary perspective on Methicillin-resistant Staphylococci: State-of-the –art-Review. *J. Vet. Emerg. Crit. Care*., **20**(1): 31–45.
34. Cremonesi, P., Luzzana, M., Brasca, M., Morandi, S., Lordi, R., Vimercati, C., Agnellin ,D., Caramenti ,G., Moroni, P. and Castiglioni, B. (2005): Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from Milk and Dairy products. *Molecular cell probes.*, **19**:299-305
35. Cunaha, M.L.R.S., Peresi, E., Calsolari, R.A.O. and Araujo, J.J.P. (2006): Detection of Enterotoxin genes in Coagulase- negative Staphylococci isolated from Foods. *Brazilian J. of Microbio.*, **37**: 64-69
36. Daka, D., Solomon, G/ S., Yihdego, D. (2012): Antibiotic-resistance *Staphylococcus aureus* isolated from Cow's milk in the Hawassa area, South Ethiopia. *Ann. Clin. Microbiol. Antimicrob*., **11**:26-37
37. David, MZ. and Daum, RS. (2010): Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. *Clin. Microbiol Rev.*, **23**:616–687.
38. De Lencastre, H., Tomasz, A.(1994): Reassessment of the number of auxiliary genes essential for expression of high-level Methicillin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemthr.,**38**: 2590–2598.
39. Deleo, F.R., and Chambers, H.F. (2009): Reemergence of antibiotic-resistant Staphylococcus aureus in the genomics era. *J. Clin. Invs.*, **119**: 2464–2474.
40. Deurenberg, R.H. and Stobberingh, E.E. (2008): The evolution of *Staphylococcus aureus. Infec. Genet Evol.*, **8**: 747-763.
41. Deurenberg, R. H., Vink, C., Kalenic, S., Friedrich, A. W., Bruggeman, C. A. and Stobberingh, E. E. (2007): The Molecular evolution of Methicillin-resistant *Staphylococcus aureus*. *Clin. Microbi. Infe.*,***13***: 222–235.
42. Dingwell, R.T., Leslie, K.E. and Duffield, T.F. (2003): Efficacy of intramammary tilmicosin and factors for cure of Staphylococcus aureus infection in the dry period. *J. Dai. Sci.*, **86**:159-168.
43. Donlan, M. and Costerton, W. (2002): Biofilms:survival mechanisms of clinically

relevant Microorganisms. *Clin. Microbiol. Rev.*, **15**:167–93.
44. Durai, R., Ng P.C.H.and Hopue, H. (2010): Methicillin-resistant *Staphylococcus aureus*: an update. *Aorn J.*, **91**(5): 599-609.
45. Ebrahimi, A., Naser, S., Somayeh, S. and Pezhman , M. (2010): Characteristics of Staphylococci isolated from mastitic goat milk in Iranian dairy herds. In: Deinhofer, M. and Pernthaner, A. (eds): Staphylococcus spp.as mastitis-related pathogens in goat milk. *Vet. World*, **3** (5): 205-208 from department of Pathobiology, School of Veterinary Science, Shahrekord University, Shahrekord, Ira
46. EFSA (2006): Scientific opinion of the panel on biological hazards on a request from the European Commission on assessment of the public health significance of methicillin resistant Staphylococcus aureus (MRSA) in animals and foods. *EFSA Journal*. **993**: 1-73.
47. Ericsson, J.M. and Sherris, J.C. (1971): Antibiotic Sensitivity Testing: report of an International Collaborative study. *Acta Pathol Microbiol Scand.*, **217**(Suppl):1-90.
48. Erskine, R. J. (2001): Mastitis control in dairy herds. In: Radostits, O. M. (ed.): Herd Health: Food and Animal Production. 3rd ed. Philadelphia: W. B. Saunders Company. Pp 397 – 435
49. European Food Safety Authority (EFSA) (2009): Assessment of the Public Health significance of Methicillin resistant *Staphylococcus aureus* (MRSA) in Animals and Foods. Scientific Opinion of the Panel on Biological Hazards. *EFSA J.*, **993**:1-73
50. European Food Safety Authority (EFSA) (2012): Scientific Report of EFSA: Technical specifications on the harmonized monitoring and reporting of Antimicrobial resistance in Methicillin-resistant *Staphylococcus aureus* in Food-producing Animals and Food. *EFSA J.*, 10:2897
51. Fall (2015): Jackie Reynolds, Richland College, *BIOL* 2421.
52. Finegold ,S.M. and E.E. Sweeney (1961): New Selective and Differential Medium for Coagulase-Positive Staphylococci Allowing Rapid Growth and Stain Differentiation. *J. Bacteriol.*, **81**:636-641.
53. Fitzgerald, J.R., Sturdevant, D.E., Mackie ,S.M., Gill ,S.R. and Musser, J.M. (2001): Evolutionary Genomics of *Staphylococcus aureus*: insights into the origin of Methicillin-resistant Strains and the toxic shock syndrome epidemic. *Proc. Natl. Acad. Sci., USA.*, **98**: 8821–8826.
54. Forbes, B. A., Sahm, A. S. and Weissfeld, D. F. (1998): Bailey and Scotts Diagnostic Microbiology, 10th Ed. Mosby, Inc., St. Louis, Mo.
55. Forsgren, A. and J. Sjoquist (1967): "Protein A" From. Reaction with Rabbit Gamma-Globulin. *J. Immunol.*, **99**:19-24.
56. Foster, J. (2005) : Immune evasion by staphylococci. *Nat. Rev Microbiol.*, **3**:948–58
57. Franco J.C., González L.V., Gómez S.C., Carrillo J.M. and Ramírez J.J.C. (2008): Virulence factors analysis of *Staphylococcus aureus* isolated from Bovine Mastitis in México. eGnosis, *ISSN.*, **6** (7): 1665-5745.
58. Francois, P., Pittet, D., Bento, M., Pepey, B., Vaudaux, P., Lew, D., Schrenzel, J. (2003): Rapid Detection of Methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile Clinical Samples by a new Molecular assay. *J. Clin. Microbiol.*, **41**(1):254–260.
59. Genta ,M.L. and Heluane, H.(2001): Biochemical Identification of most frequently encountered Bacteria that cause Food spoilage. In: Spencer, J. T. and Ragout de Spencer, A. L. (eds). Food Microbiology Protocols. Humana Press Inc. *Totowa, New Jersey.*, **14**: 3-11.
60. Getahun, K. (2006): Bovine mastitis and antibiotic resistance patterns of major pathogens in small Holder dairy farms in the centeral highlands of Ethiopia. Msc. Thesis, Addis Ababa University, Fucalty of Veterinary Medicine, Debr-Zeit, Ethiopia.
61. Gill, R., Howard, W.H., Leslie, K.E. and Lissmore, K. (2005): Economics of mastitis control. Dai. Sci., **61**:27-43.
62. Gordon, J. and Lowy, D. (2008): Pathogenesis of methicillin resistant *Staphylococcus* aureus infection. *Clin. Infec. Dis.*, **46**:350–359.
63. Green ,M. and Bradley ,A. (2004): *Staphylococcus aureus* mastitis in Cattle. Clinical Forum, UK. *VET. J.*, **9** (4):1-9.
64. Harmsen, D., Claus, H., Witte, W., Rothganger, J., Turnwald, D. and Vogel, U. (2003): Typing of Methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for Spa repeat Determination and Database management. *J. Clin. Microbiol.*, **41**:5442-5448.
65. Harris, G., Foster, S. and Richards ,G.(2002): An Introduction to *Staphylococus aureus* and techniques for identifying and quantifying *Staphylococcus aureus* adhesions. *Review*., **4**:39-60.
66. Herold, B.C.,Immergluck, L.C., Maranan, M.C., Lauderdale, D.S., Gaskin, R.E., Boyle-Vavra, S.,

Leitch, C.D. and Daum, R.S. (1998): Community-acquired Methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA: *the J. of the American Medical Associa.., **279***: 539-598.

67. Hu, C.J., Fang, R.H., Luk, B.T., Zhang, L. (2013): Nano particle-detained toxins for safe and effective vaccination Nat. *Nano. Technol.,* **8**: 933-938
68. Huber, H., Giezendanner, N., Stephan, R. and Zweifel , C. (2011): Genotypes, antibiotic resistance profiles and microarray-based characterization of methicillin-resistant Staphylococcus aureus strains isolated from livestock and veterinarians in Switzerland. *Zoo. Pub. Hlth.,* **58:**343–49.
69. ISO/TS 6888-3. (2003): Microbiology of Food and Animal Feeding Stuffs – Horizontal method for the Detection and Enumeration of Staphylococcus spp. Part 1: Detection method. International Organisation for Standardisation (ISO), ISO Central Secretariat, 1 rue de Varembé, Case Postale **56:** CH - 1211, Geneva 20, Switzerland.
70. Jablonski, M. and Bohach, A. (1997): *Staphylococcus aureus.* Food Microbiology Fundamentals and Frontier, ASM Press, Washington, D.C., USA, pp 353-375.
71. Jayarao, B.M. and Cassel ,E.K. (1999): Mastitis prevention and Milk hygiene practices adopted by Dairy practitioners. *Large Anim. Pract.,* **20**:6–14.
72. Jevons, M.P. (1961): 'Celbenin'-resistant Staphylococci. *British Medical J.,* **1**:124125.
73. Jones, F., Kellum, E., Porter, S., Bell, M. and Schaffner, W. (2002): An outbreak of community acquired food borne illness caused by methicillin-resistant Staphylococcus aureus. *Emerg. Infe. Dis.,* **8**:82–84.
74. Jorgensen ,J.H. and Turnidge, J.D. (2007): Antibacterial Susceptibility tests: Dilution and Disk Diffusion methods. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. Manual of Clinical Microbiolog. 9th ed. Washington, DC: American Society for Microbiology., p. 1152-72.
75. Joshi, L.R., Tiwari, A., Devkota, S.P., Khatiwada, S., Paudyal, S. and Pande, K.R. (2014): Prevalence of methicillin-resistant Staphylococus aureus (MRSA) in dairy farms of Pokhara, Nepal. Inter. *J. Vet. Sci.,* **3**:87-90. www.ijvets.com.
76. Juhasz-Kaszanyitzky, E., Janosi, S.,Somogyi, P., Dan, A.,Van derGraaf-van Bloois, L., Van Duijkeren ,E.and Wagenarr,J.A. (2007): MRSA transmission between Cows and Humans. *Emerg. Inf. Dis.,* **13**:630-632.
77. Kerro, D. and Tareke, F. (2003): Bovine Mstitis in selected areas of Southern Ethiopia. Trop. *Anim. Health Prod.* **35**:197-205.
78. Khanna, T., Friendship, R., Dewey, C. and Weese, J. S. (2008): Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. *Vet. Micro.*,**28**:298-303.
79. Kloos ,W.E., Bannerman ,T.L., Murray, P.R., Baron, E.J., Pfaller ,M.A., Tenover, F.C. and Yolken, R.F. (1995): Staphylococcus and Micrococcus. In: Manual of Clinical.
80. Kluytmans, A.(2010): Methicillin-resistant *Staphylococcus aureus* in Food products: cause for concern or case for complacency? *Clin. Microbiol. Infect.,* **16**(1):11–15.
81. Kwon, N.H., Kun, T.P., Jin, S.M., Woo, K.J. and So, H.K.(2005): Staphylococcal Cassette Chromosome mec (SCCmec) Characterization and Molecular analysis for Methicillin resistant *Staphylococcus aureus* and novel SCCmec subtype IVg isolated from bovine milk in Korea. *J. Antimicrob. Chemother.,* **56**:624–632.
82. Lee, J. (2003): Methicillin (Oxacillin)-resistant Staphylococcus aureus strains isolated from major food animals and their potential transmission to humans. *Appl. Environ. Microbiol.,* **69**: 6489- 6494.
83. Leonard, F.C. and Markey ,B.K.(2008): Methicillin-resistant *Staphylococcus aureus* in animals: *A rev.Vet.J,.* **175**: 27–36.
84. Levinson,W. (2008): Medical Microbiology and Immunology. 10th ed., McGraw-Hill Companies, Inc. San Francisco, California.
85. Lourdes, M.,Cunha,R., Sinzato, Y. and Silveira,L. (2004): The Medical significance and Identification of Coagulase negative Staphylococci. J. Clin. Microbiol.,**99**: 855-860.
86. Lowy, D. (1998): *Staphylococcus aureus* infections. N. Engl. *J. Med*., **339**:520–532.
87. MacFaddin,J.F. (1985): Media for isolation-cultivation-identification-maintenance of medical bacteria, 1st ed. Williams & Wilkins Co., Baltimore, MD., 1: 263, 535, 540, 564, 620.
88. Mahon, C. R. and Manusekis ,G. (1995): Textbook of diagnostic microbiology. W.B. Saunders Company, Philadelphia, P 106
89. Mahon, C.R. and Larsen, H.S. (1995): *Staphylococcus aureus*. In Textbook of diagnostic microbiology. Edited by Mahon C.R., Manuseseilis G Jr. New York, WB. Saunders Company, Pp. 325-33
90. Manian, F.A. (2003): Asymptomatic nasal carriage of mupirocin-resistant methicillin-

resistant *Staphylococcus aureus* in a pet dog associated with MRSA infection in household contacts. *Clin. Infect. Dis.*, **36**: 26-28.
91. Matsunaga , T., Kamata, S., Kakiichi, N. and Uchida, K. (1993): Characteristics of *Staphylococcus aureus* isolated from per acute, acute and chronic bovine mastitis. *J. Vet. Med. Sci.*, **55**: 297-300.
92. McCarthy, N.L., Sullivan, P.S., Gaynes, R. and Rimland, D.(2010): Health careassociated and community-associated methicillin-resistant *Staphylococcus aureus* infections: a comparison of definitions. *Am. J. Infe. Contr.*, **38**:600–606.
93. McCormick, K., Yarwood, M. and Schlievert, M. (2001): Toxic shock syndrome and bacterial superantigens: an update. *Annu. Rev. Microbiol.*, **55**:77–104.
94. McCormick, K., Yarwood, M. and Schlievert, M. (2001): Toxic Shock syndrome and Bacterial Superantigens: an update. *Annu. Rev. Microbiol*., **55**:77–104.
95. Menzies, E.(2003): The role of fibronectin binding proteins in the pathogenesis of Staphylococcus aureusinfections. *Curr. Opin. Infect. Dis.*, **16**:225–9.
96. Montanari, M.P., Tonin, E., Biavasco, F. and Varaldo, P.E. (1990): Further Characterization of borderline Methicillin-resistant *Staphylococcus aureus* and analysis of Penicillin-binding proteins. *Antimicrob Agents Chemother*., **34**:911– 913.
97. Mooij, T.A., Jenkins, J. and Thijssen, S. (2007): MRSA in calves. *Infec. ieziekten Bull*, **18**:234-236.
98. Morgan, M.(2008): Methicillin-resistant *Staphylococcus aureus* and Animals: Zoonosis or Humanosis? *Journal of Antimicrobial Chemotherapy.*, **62**(6):1181-1187.
99. Morrison, R.(2008): The Coagulase test in the Identification of Pathogenic Staphylococci. *J. Appl. Bacteriol.*, **25**: 432-435.
100. Mullarky, I.K., Wolfe, P.C.S.and Jones, G.M.(2010): *Staphylococcus aureus* Mastitis: Cause, Detection, and Control. Paper revi., Virginia cooperative Extension, Virginia Polytechnic Institute and State University., Pp 404-229.
101. NMSA (National Meteorological Services Agency), (2014): Monthly report on temperature and Rainfall Distribution for Asosa Zone, Regional Metrological Office, Asosa, Ethiopia.
102. National Centre for Biotechnology Information (NCBI): Taxonomy browser (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1279). Accessed 21, January 2014.
103. National Committee for Clinical Laboratory Standards (2011): Performance standards for antimicrobial susceptibility testing, 7th informational supplement. Approved standard M100-S21.
104. National Mastitis Council (NMC) (2004): Procedures for Collecting Milk Samples. In: Microbiological Procedures for the Diagnosis of Bovine Udder Infection and Determination of Milk Quality, NMC publication.
105. NCCLS (2012): Performance standards for antimicrobial susceptibility testing. Thirteenth informational supplement. Approved standard M100-S13. National Committee for Clinical Laboratory Standards, Wayne, PA
106. NMC (1990): National Mastitis Council (NMC) Microbiological procedures for the diagnosis of bovine udder infection. 3rd ed., Madison, Wisconsin: National Mastitis Council Inc., Pp 7-8.
107. Normanno, G., Corrente, M., La Salandra ,G *et al*. (2007): Methicillin resistant *Staphylococcus aureus* (MRSA) in Foods of Animal origin produced in Italy. *Int. J. Food Microbiol.* **117**(2):219-222.
108. Novick, P. (2003): Autoinduction and signal transduction in the regulation of staphylococcal virulence. *Mol. Microbiol.*, **48**:1429–49
109. O'Mahony, R., Abbott, Y., Leonard, F.C., Markey , B.K., Quinn, P.J., Pollock, P.J., Fanning, S. and Rossney, A.S.(2005): Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from Animals and Veterinary personnels in Ireland. *Vet. Microboiol.*, **109**: 285– 296.
110. O'Riordan, K. and Lee, C.(2004): *Staphylococcus aureus* Capsular polysaccharides. *Clin. Microbiol. Rev*., **17**:218–34.
111. Owens, W.E., Ray, E. H., Watts, J. L. and Yancey, R. T. (1997): Comparison of success and results of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis, *J. Dai. Sci.*,**80**: 313-317.
112. Pal, M. (2007): Zoonoses. 2nd ed. Satyam Publishers, Jaipur, India., Pp. 138-139.
113. Pal, M. and Seid. (2013): Resent Diagenostic approches for Staphylococcal Food poisoning Beverage and Food World., **40**: 43-44

114. Paterson, K., Larsen, J., Harrison, M., Larsen , R., Morgan, J., Peacock, J., Parkhill, J., Zadoks, N. and Holmes, A. (2012): First detection of livestock-associated meticillin-resistant *Staphylococcus aureus* CC398 in bulk tank milk in the United Kingdom. Euro. Surv., 17:20337.
115. Patti, J.M., Allen, B.L., McGavin, M.J. and Hook, M. (1994): Microbial Surface components recognizing adhesive matrix molecule (MSCRAMM)-mediated adherence of microorganisms to host tissues. *Annu. Rev. Micro.*, **48**:585–617
116. Peacock, J., de Silva, I. and Lowy, D. (2001): What determines nasal carriage of *Staphylococcus aureus*? *Trends Microbiol.*, **9**(12):605–10
117. Persoons D., Van Hoorebeke S., Hermans K., Butaye P., de Kruif A., Haesebrouck F. and Dewulf J. (2009): Methicillin-resistant Staphylococcus aureus in poultry. *Emerg. Infect Dis.*, ***15***:452-453.
118. Pesavento, G., Ducci , B., Comodo , N. and L. Nostro, A. (2007): Antimicrobial resistance profile of Staphylococcus aureus isolated from raw meat: A research for methicillin resistant Staphylococcus aureus (MRSA). *Food Cont.*, **18**:196-200.
119. Petersson- Wolfe, C.S., Mullarky, I.K. And Jones ,G.M. (2010): *Staphylococcus aureus* mastitis: Cause, Detection, and Control. Virginia cooperative extension, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University., Pp 404-229.
120. Philpot, W.W. (1979): Control of mastitis by hygiene and therapy. *J. Dai .Sci.*, **62**:168176.
121. Prevost, G., Couppie, P. and Monteil,H. (2003): Staphylococcal Epidermolysins. *Curr. Opin. Infect. Dis.*, **16**:71–6.
122. Proctor, A. and Peters, G. (1998): Small colony variants in Staphylococcal infections: Diagnostic and therapeutic implications. *Clin. Infect. Dis.*, **27**:419–22
123. Pyorala, S. (2003): Indicators of inflammation in the diagnosis of mastitis. *Vet. Res.*, **34**:565-78.
124. Quinn, p.J., Carter, M.E., M.E Markely, B. and Carter, G.R.(1999): Clinical Veterinary Microbiology Mosby International Limited, Spain., PP118-143,209-242.
125. Quinn ,P.J., Carter, M.E., Donnelly, W.J.C. and Leonard, F.C.(2002): Veterinary microbiology and microbial disease. Blackwell science, London Pp.465-472
126. Radostits, O.M., C.C. Gay., D.C. Blood. and Hinchlif, K.W.(2007): Mastitis. In: Veterinary medicine, 9th ed. Harcourt Ltd, London, Pp 174-758.
127. Randolph, T.F., Schelling, E., Grace, D., Nicholson, C.F., Leroy,J. L., Cole, D. C., Demment, M. W., Omore, A., Zinssstag, J. and Ruel, M.(2007): Role of Livestock in Human nutrition and health for poverty reduction in Developing countries. *J. of Animal Scien.*, **85**: 2788-2800.
128. Resch, M., Nagel, V. and Hertel, C. (2008): Antibiotic resistance of coagulase-negative staphylococci associated with food and used in starter cultures. *Int. J. Food Microbiol.*, **127**:99-104
129. Resch, M.,Nagel, V. and Hertel, C.(2008): Antibiotic resistance of Coagulase-negative Staphylococci associated with Food and used in starter cultures. *Int. J. Food Microbiol.*, **127**:99-104.
130. Rho, M. and Schaffner, D. (2007): Microbial risk assessment of Staphylococcal Food poisoning. *Inter. J. of Food Microbiol.*, **116**: 332-338.
131. Roberson, J.R., Fox, L.K., Hancock, D.D., Gay, J.M. and Besser, T.E. (1998): Sources of intramammary infections from Staphylococcus aureus in dairy heifers at first parturition. *J. Dairy Sci.*, **81**: 687-93.
132. Roberson, R. (1999): The Epidemiology of *Staphylococcus aureus* on Dairy farms. In: Proceedings of the 1999 Annual Meeting of the National Mastitis Council. National Mastitis Council, Inc., Madison, W.I., P38.
133. Rota, C., Yanguela, J., Blanco, D., Carraminana, J., Arino, A. and Herrera, A. (1996): High prevalence of multiple resistances to antibiotics in 144 Listeria isolates from Spanish dairy and meat products. *J. Fo. Prot.*, **59**: 938–943.
134. Rowe, J. (1999): Milk quality and Mastitis. In: Small Ruminant for mixed practitioner. Western Vet. Confe., Lasvagas., Pp 152-156.
135. Russel, C.D. (2012): *Staphylococcus* Vaccination and MRSA. Lancet Infect. Dis., 12: 586
136. Rychlik, I., Gregorova, D. and Hradecka, H. (2006): Distribution and function of plasmids in Salmonella enteric. *Vet. Microb.*, **112**: 1-10.
137. Sandel, M.K. and McKillip. (2004): Virulence and recovery of *Staphylococcus aureus* relevant to the Food industry using improvements on traditional approaches. Food Content, 15: 510.
138. Schito, G. (2006): The importance of the development of antibiotic resistance in

Staphylococcus aureus. *Clin. Microbiol. Infect.*, **12**:3–8.
139. Schukken,Y.H.,Wilson,D.J.,Welcome,F.,Garrison-Tikofsky,L.andGonzalez,R.N.(2003): Monitoring udder health and milk quality using somatic cell counts.*Vet.Res.*,**34**:579-596.
140. Sears, P.M. and McCarthy, K.K. (2003): Management and treatment of staphylococcal mastitis. Vet. Cl. North Am. *Food An. Pra.*, **19**:171-185.
141. Sharma, D., Kumar, P. and Malik, A. (2011): Prevalence and Antimicrobial Susceptibility of Drug Resistant Staphylococcus aureus in raw milk of dairy cattle. *Int. Res. J. Microbiol.*, 2:466-47.
142. Silva, W., Destro, M., Landgraf ,M. and Franco, B. (2000): Biochemical characteristics of typical and atypical Staphylococcus aureus in mastitic milk and environmental samples of Brazilian dairy farms. *Brazilian J. Microbiolo.*, **31**: 103-106.
143. Simoons-Smit, A.M., Savelkoul,P.H., Stoof, J., Starink, T.M.and Vandenbroucke-Grauls, C.M. (2000): Transmission of *Staphylococcus aureus* between humans and domestic animals in a household. Eur. *J. Clin. Microbiol. Infect. Dis.*, **19**:150–225
144. Stevens, D.L. (2004): Optimizing outcomes in Methicillin_resistant *Staphylococcus aureus* infections: focus on nosocomial pneumonia and SSTI, highlights from a satellite symposium at the 11th annual international congress on infectious disease (ICID), *Cancun, Mexico.*, Pp 1 - 8.
145. Tamirat, T. (2007): Comparison of clinical trials of bovine mastitis with the use of honey. MSc thesis, Addis Ababa University, Ethiopia, Pp 14-30
146. Tenover, C. and Goering, V. (2009): Methicillin-resistant Staphylococcus aureus strain USA300: origin and epidemiology. *J. Antimicrob. Chemo.Ther.*, **64**:441-446.
147. Thirapatsakun, T. (1999): Mastitis management. In: small holder dairying in the tropics. In: Hunt and Chantalakhana (Eds). ILRI, Kenya, Nairobi, 299-339.
148. Thrusfield, M. (2005): Veterinary Epidemiology. 3rd edition. Blackwell Science Ltd., UK., Pp 233-250.
149. Turutoglu, H., Tasci, F. and Ercelik, S. (2005): Detection of Staphylococcus aureus in milk by tube coagulase test. *J. Appl. Microbiol.*, **49**: 419-422.
150. Van Loo, I., Huisdens, Tiemersma, E., de Neeling, A.,van de, Sande-Bruinsma, N., Bearijlan,D.,VossVandenesch,F.,Naimi,T.,Enright,M.C.,Lina,G.,Nimmo,G.R.,H effernan,H.,et al.(2003):Community-acquiredmethicillin-resistant Staphylococcus aureus carryingPanton-Valentine leukocidin genes: worldwide emergence. *Emerg.. Infec. Dis.*, **9**: 978–984.
151. Vanderhaeghen, W., Hermans, K., Haesebrouck, F. and Butaye, P. (2010): Methicillin-resistant *Staphylococcus aureus* (MRSA) in Food production Animals. *Epidemiol. Infect.*,**138**: 606–625.
152. Vannuffel, P., Laterre,P.F., Bouyer, M., Gigi, J., Vandercam, B., Reynaert, M., Gala, J.L.(1998): Rapid and Specific Molecular identification of Methicillin-resistant *Staphylococcus aureus* in endotracheal aspirates from mechanically ventilated patients. *J. Clin. Microbiol.*, **36**(8):2366–2368.
153. Voss, A., Loeffen, F., Bakker, J., Klaassen, C. and Wulf, M.(2005): Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg. Infect. Dis.*, **11**: 1965–1966.
154. Walderhaug, M. (2007): Food borne pathogenic microorganisms. Center Food Saf. App. Nuit., **28**: 48-65.
155. Walderhaug, M. (2007): Foodborne pathogenic microorganisms and natural toxins. Food and Drug Administration, Center for Food Safety and Applied Nutrition, **28**: 48-6
156. Walther, B., Wieler L.H., Friedrich A.W., Hanssen, A.M., Kohn ,B., Brunnberg, L., LubkeBecker, A. (2008): Methicillin-resistant Staphylococcus aureus (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. *Vt. Micrb.*, **127**(1-2): 171-178.
157. Walther ,C., Perreten, V. (2007): Methicillin-resistant *Staphylococcus epidermidis* in organic milk production. *J. Dairy Sci.*, **90**(12):5351.
158. Wang, Y., Wu, C.M., Lu, L.M., Ren, G.W.N.,Cao, X.Y. and Shen, J.Z.(2008): Macrolidelincosamide resistant Phenotypes and Genotypes of *Staphylococcus aureus* isolated from Bovine Clinical Mastitis. *Vet. Microbiol.*,**130**: 118–125.
159. Witte, W., Strommenger, B., Stanek, C., Cuny, C.(2007): Methicillin resistant Staphylococcus aureus ST398 in humans and animals, Central Europe. *Emer. Inf. Dis.*, **13**:255-25.
160. Workeneh, S., Bayleygne, M., Mekonnen, H. and Potgieter, L. (2002): Prevalence and etiology of mastitis in cows from two major Ethiopian dairies. *Trop. Anim. Hlth.. Prod.*, **34**:19-2.

161.

12/2/2024