BOVINE MASTITIS: PREVALENCE, RISK FACTORS AND ISOLATION OF STAPHYLOCOCCUS IN LACTATING DAIRY COWS AT ABRAHAMO WOREDA, WESTERN ETHIOPIA

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ABSTRACT: Across-sectional study was conducted from September 2024 to Febuarary, 2025, on the Bovine mastitis: Prevalence, risk factors and Isolation of Staphylococcus aureus in Dairy cows at Abrahamo Dsistrict, western Ethiopia, with the objectives of estimating the prevalence of Staphylococcus, and its associated risk factors**.** A total of 365 milk samples were collected with simple randon sampling techniques and kebeles were selected purposive as convenient. The overall prevalence of mastitis at cow level was 118/365 (32.3%). 35(22.87%) and 83(39.2%) of clinical and subclinical mastitis prevalence were identified. Similarly, 21(13.72%) and 76(35.84%) of clinical and sub clinical Staphylococcus aureus prevalence were investigated. 40.22% and 24.30% prevalence of mastitis were seen in cross and local breeds respectively, which were significant(P<0.05). Age, breed, parity, milking hygiene, pregnancy status, teat lesion, blind teat, and udder shape risk factors showed significant value for the prevalence of mastitis in the study area (P<0.05) while the floor system, origins, previous mastitis history and treatment history and Stage of lactation were non-significant. The present result showed a significant association of resistance pattern with *S.aureus* isolates, particularly to Penicillin G(94.7%), Cefoxitin (86.84%), and kanamycin (100%). In this study, *S. aureus* were found to be highly susceptible to Doxycycline (94.73%), Ciprofloxacillin (92.1%), Gentamycin (92.1%), Trimethoprim-sulfamethoxazole (81.6%), Tetracycline (73.68%), and followed by Ampicillin (73.68%). 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. Hence, Dairy farm mastitis and Staphyloccoccus prevention and control methods will be done so as reduce the risk and increase production and productivity of milk.

[*Asmamaw Aki Jano and Mitiku Belete.* **BOVINE MASTITIS: PREVALENCE, RISK FACTORS AND ISOLATION OF STAPHYLOCOCCUS IN LACTATING DAIRY COWS AT ABRAHAMO WOREDA, WESTERN ETHIOPIA.** *Life Sci J* 2025;22(8):35-49]. ISSN 1097-8135 (print); ISSN 2372-613X (online)**.** http://www.lifesciencesite.com**.** 04. doi:10.7537/marslsj220825.04

Key words*: Abrahamo; Bovine; mastitis; Staphyloccocus; risk factors*

1. INTRODUCTION

Ethiopia has the largest livestock population in Africa, estimated at 70 million cattle (CSA, 2020/21). Mainly, they serve as a source of food and income that milk and milk products support about 50–70% of the rural people(Duguma, 2020). In Ethiopia, more than 80% of milk for human consumption is obtained from cattle unity. However, milk production often does not meet national requirements due to, the extensive, low inputs of animal husbandry practices, and widespread livestock diseases. The quality and quantity of milk in the country deteriorates due to various causes among them udder infection is the one(Minten *et al.*, 2020).

Mastitis is inflammation of mammary gland, primary resulting from invasion of the mammary gland by pathogenic microorganisms through the teat canal resulting in physical, chemical, pathological and bacteriological changes in glandular tissues and milk (Zigo *et al.*, 2021). The disease is a highly prevalent problem in dairy cattle and is one of the most important threats affecting the world's dairy industry. Bovine mastitis can be clinical with local (in some cases general) clinical signs and milk abnormalities or sub clinical with production losses and lowered milk quality (Neculai-Valeanu and Ariton, 2022). Several etiological agents cause mastitis of which *S.aureus* is a major pathogen that also poses food safety and antimicrobial resistance threats (Neelam *et al.*, 2022). *Staphylococcus aureus* is the most predominant cause of mastitis in dairy cows in Ethiopia. The antibiotic resistance characteristics of the bacterium have been the main concern of the world as far as the control of infections caused by pathogenic strains of S. aureus is concerned (Tezera and Aman Ali, 2021). The disease results from the interaction of various factors associated with the host, pathogen(s), environment and management Virulence factors ,such as adhesion factors, and collagenase secretions, are linked to the pathogenicity of *S. aureus* (Altin *et al*., 2019).

The Bacterial contamination of milk from an infected udder may render it unsuitable for human consumption by causing food poisoning or providing a vehicle for the spread of zoonotic diseases to humans (Mokgaotsi, 2019). Resistant bacteria from food animals may be passed through the food chain to humans resulting in resistant infections (Khairullah *et al.*, 2020).The major sources of pathogens and means of transmission include infected quarters and soiled udder, contaminated milking machines or milker's hands, udder washing trend, use of drying clothes, and tick infestation. Improvement of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country by controlling some of the major infectious disease through regular monitoring (Khairullah *et al*., 2020).

Statement of the Problem: The majorities of Ethiopia's new dairy farms' management systems remained traditional and lack optimal management methods and are prone to mastitis. Milk is a major component in the human diet all over the world, but it also serves as a good medium for the growth of many microorganisms, especially pathogenic bacteria (Wesolowska *et al.*, 2019). Pathogenic organisms in milk can be derived from the cow itself, the human hand, or the environment(Bedane A *et al.*, 2012)**.**

In Ethiopia, currently there is a national interest to improve the dairy sector to enhance the benefits that would be gained from this sector. In line with this, animal disease researches that are directly related to dairy production help as an input for any intervention. In Ethiopia, *Staphylococcus* species isolated from animal origin food in various parts of the country indicate the presence of an alarming level of resistance isolates (Tsehayneh *et al*., 2021). Similarly, there was studies conducted on isolation, identification, and prevalence associated risk factors, of *S. aureus* in lactating dairy cow's milk in Benishangul Gumuz; such as of Asossa district, the mastitis prevalence of 28.4%, at the study area was reported by (Asmamaw A, *et al*. 2017**).** So the present research indicates the higher prevalence of mastitis (32.3%) and 26.57% of Staphylococcus prevalence in the Abrahamo woreda, which implies as the Dairy cows' mastitis control and prevention measures were in -effective and the infection was not reduced rather rating and hence milking hygienes and control options will be done with concerned stalkholders so as to control and enhance the production. And the potential risk factors was managed by animal health workers and animal owners. Therefore, the objective of this study were to determine the prevalence of mastitis in Dairy cows in Abrahamo District, to isolate and identify *Staphylococcus* from mastitis positive cows, and to assess the risk factors associated with bovine mastitis and *Staphylococcus* infections.

2. METHODOLOGY

2.1 Study Area

Abrahamo woreda is one of the Assosa zone woreda in Benishangul-Gumuz Regional State which is located in the North-west part of the capital city of the region. The district is located between 8030'' and 40°27" N and 34°21" and 39°1" E. Woreda has 180 altitudinal differences between the highest and the lowest places. The lowest point is 1461 meters a.s.l while the highest peak is 1641 meters above sea level (a.s.l). It has two major physiographic divisions. The first one is the warm temperate agro-climatic zone (1500-1900m), which comprises low plateaus of the Woreda and covers about the 98.49% of city land surface. The second is lowlands (less than 1500m) constitute about 1.51% of the total land area of the city. The temperature ranges from 20° C - 35° C (highest) to 12° C - 20° C (lowest). February to May is the hottest months while November to December is the cold months). The total amount of rain fall recorded at District during the last nine months of (2020) is 1,119 mm (BGRSMSC, 2020).

The rainy season starts from April/May up to October/November with an average annual rainfall that ranges from 800 mm to 2000 mm. The rainy season starts in March and extends to November with the highest concentration in June, July, and August. The livelihood of the society largely depends on mixed livestock and crop production. Majority the people of Woreda are merchants; about 17 percent are agrarian especially in animal rearing such as cattle, sheep, goats, donkeys, mules, and poultry and the rest participate small and medium scale industries(CSA & BoFed, 2007 & 2012). Extensive livestock husbandry, feeding and outdoor housing system was found, Back yard chicken Management system is practiced, where local breeds are allowed to scavenge, what nature provides them. In the woreda, animal movement was due to agriculture and trade purpose. Animal in the area was used for meat, milk, ploughing, traction power, and income generation. Socioeconomy of the people in the area was mainly depending on mixed farming (CSA, 2015 and Abrahamo woreda agriculture office, 2021).

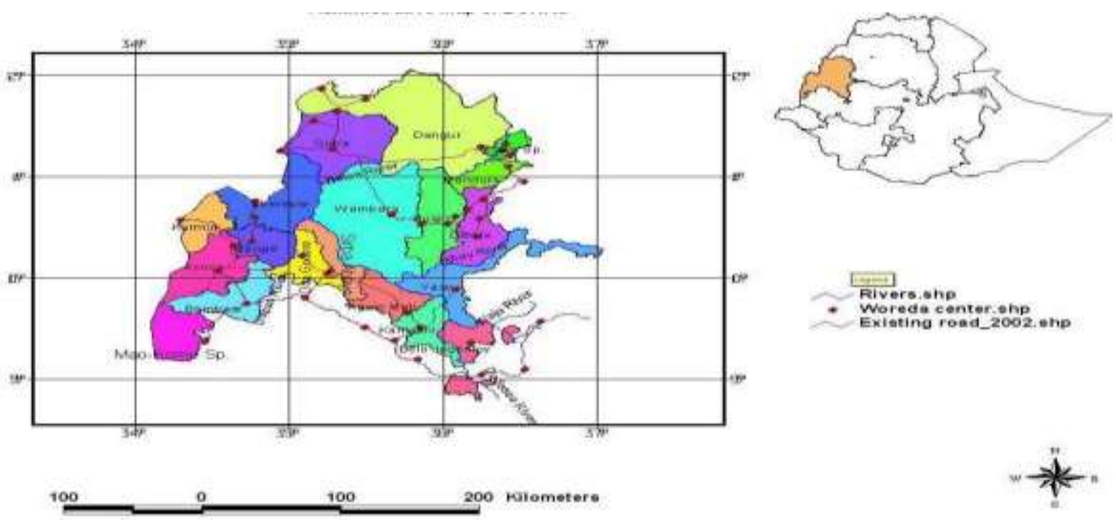

Figure: 1. Administrative maps of BGRS, 2015

2.2. Study Design and Sampling period

A cross-sectional study was conducted from September 2024 to Febuarary, 2025, to determine the prevalence of mastitis, staphylococcus and associated risk factors in the study area. After proper clinical examination of the udder of the cows on the selected farms, cows showing clinical signs of mastitis are randomly sampled as the aim were determining the prevalence of mastitis and *Staphylococcus,* to know its status on the occurrence of mastitis.

2.3. Study Population

The study population was dairy cows found in Abrahamo woreda of Asossa zone. The study includes, Bovine lactating cows of both breeds, cross breed and local Zebu breeds, which are managed under small farmstead and individual animal lactating cows and household who keep cattle in selected district.

2.4 Sample size Determination

The sample size for lactating cow was determined according to Thrusfield (2005) using 95% confidence interval, 5% absolute level of precision and expected prevalence of 39.32% of mastitis which was reported by (Asmamaw A, *et al*. 2017) previously at study area of Asossa zone of Asossa town. Hence, the sample size was calculated using the formula given here below. Therefore, the total sample size for the study were calculated as follows:

$$n = \frac{(1.96)^2 x\ P\ (1-P)}{d2}$$

Where: n = the total sample size, P = expected prevalence (39%)

d = desired absolute precision (5%)

(0.05) at 95% CI ; n = (1.96 x 1.96) x (0.39) x (1-0.39)/((0.05) x (0.05) = 365 samples.

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2.5 Sampling Procedure, Collection and Handling

The milk sample was taken from cows not treated previously with either intra mammary or systematic antimicrobials agents. Aseptic procedure was followed during milk samples collection in order to prevent contamination with microorganisms 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 contaminations of teat canal (Quinn, *et a*l., 2002). Sterile universal bottle with tight fitting cups was used. The universal bottle was labelled 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(Hemling *et al*., 2002) .

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 (Hora and Ebissa, 2021). After milking out and discarding the first two drops, about 2ml of milk was tested on CMT paddle. After CMT test, the sample taken from each quarter and about 20 ml of milk was aseptically collected from mastitis positive result using sterile universal bottle. Finally, by using Ice box, the collected samples was transported to Asossa, Regional Veterinary Laboratory and submitted immediately to Veterinary microbiology laboratory within 24 hrs for microbiological examination. The samples was kept at 4ºC overnight before culturing.

2.6 Questionnaire survey

During sample collection, all information related to each sampled cow was gathered by using semi structured questionnaire. Data regards to the animal identification, the farm management and all about potential risk factors for the occurrence of mastitis in dairy cows was collected through physical observation and by interviewing the farm owners or handlers. The animal level factors such as presence of teat lesion, teat blindness, body condition, parity, lactation stage, breed and age difference was recorded. The farm level factors such as herd size, housing types, farm hygiene, previous history of treatment of mastitis, barn floor status, type of milking method, use of towels, milking sequences and hygiene was recorded. Udder and milk abnormalities (injuries, blindness, tick infestation, swelling, milk clots, abnormal secretion, etc.) was also be recorded.

2.7 Clinical Inspection of the Udder

To identify a clinical mastitis, the udders of the cows was examined by visual inspection and palpation for the presence of any lesion, pain, heat, and swelling. In addition, milk from each quarter is withdrawn and checked for any change in colour and consistency. These mastitis cases are diagnosed on the basis of manifestation of visible signs such as inflammation of the udder characterized by heat and swelling with pain upon palpation and/or gross changes in milk. Additionally its examined when misshaped, atrophied, hard, and fibrotic quarters are observed(Quinn *et a*l.,2002).

2.8 California Mastitis Test (CMT)

The California mastitis test was conducted to diagnose the presence of sub clinical mastitis and it was carried out according to standard procedures. A squirt of milk from each quarter of the udder was 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 . If at least one quarter was positive by the CMT then the cow was considered as positive (Quinn *et al*., 2002)

2.9 Laboratory Analyses

Cultural Methods *:* The samples collected from cows were cultured on general purpose media such as blood agar and nutrient agar using a sterile loop inside the biosafety cabinet and around the Bunsen burner. Other selective and differential media such as Mannitol salt agar used for cultural purposes. Inoculated plates will be incubated aerobically at 37°C. After 24 hours of incubation, the plates are removed from the incubation and examined visually. Any growth, pigmentation, hemolysis, and colonial morphology was noted accordingly(Kasa *et al*., 2020).

Bacterial Isolation and Identification *:* The milk samples were streaked onto blood agar base enriched with 7% sheep blood. The plates were aerobically incubated at 37ºC and examined for bacterial growth after 24-48 hours. From culture positive plates, typical colonies was subjected to Gram's stain to study their gram reaction and cellular morphology. Similar colonies of pure culture was transferred from blood agar into nutrient agar plate to get fresh colonies.

Using gram stain reaction and biochemical Tests, Colonies representative of each type of bacterium was stained by Gram's method and then examined microscopically for Gram staining reaction (positive staining purple or negative staining pink), size (small, medium, or large), and shape (rods, cocci, or coccobacilli). Further characterization of the isolates was by using conventional biochemical tests (Catalase, Oxidase, Indole production, methyl red test, Voges–Proskauer test, Citrate utilization, Triple sugar iron, and coagulase tests) (Traverso *et al.*, 2010).

2.10 Antimicrobial Susceptibility Test

Antimicrobial resistance pattern of the S*taphylococcus* species isolates were performed on Mueller Hinton agar by the Kirby Bauer disk diffusion method using standard procedure of the Clinical and Laboratory Standards Institute (CLSI, 2014). The antimicrobials tested for resistance pattern in this study was those which proved to be often available and routinely used in the study areas for the treatment of animals. About 2-3 pure colonies of the isolates were taken from the Nutrient agar and suspended in Tryptose Soya Broth and then, incubated at 37℃ for 1-2hrs. The suspension was, then checked for the development of slight turbidity, against 0.5 MacFarland solutions and it was inoculated, by dipping a sterile cotton swab into it, and wiping on the Muller Hinton agar, according to the standard procedure (CLSI, 2014)

The streaking procedure was repeated two more times, and the plate was rotated approximately 60°C each time to ensure an even distribution of inoculum. Thereafter, different antimicrobial discs was distributed evenly on the MH plate, so that they were no closer than 24 mm from centre to centre, by sing sterile forceps and gently pressed down with the point of a sterile forceps to ensure complete contact with the agar surface. Plates were, then incubated at 37°C for 18-24 hours. After the final incubation time, the zone of inhibition around each disc was measured, with the help of a calliper, which was held on the back of the inverted petriplate and the results was interpreted as sensitive, intermediate and resistant using a standard zone of interpretative chart (CLSI, 2014).

3. Ethical Approval Consideration

Owners was convinced that the study was not harm their animals. Proper cautions was followed while sample collection and restraining procedures are taking place. So, all data collection activities was depend on voluntary responses from the society. In addition, appropriate sample size was used to determine this prevalence associate risk factors in the study. These actions have been reviewed and approved by board of JUCAVM research and academic affairs.

4. Data Analysis

The data was recorded in the Microsoft Excel Spreadsheet 2010 and coded before statistical analysis. The association among variables collected during this study time was computed using various statistical tools in STATA version12 and assessed using multivariable logistic regression analysis. A cow are denoted as positive for mastitis if at least a single teat with clinical mastitis or a CMT-positive result was detected. Accordingly, descriptive statistics such as percentages and frequency distribution are used to describe/present bacterial isolates(Dohoo *et al*., 2009).

5. RESULTS

5.1. Prevalence of mastitis

In this cross- sectional study, out of the total Dairy cows examined, 118/365(32.33%) mastitis prevalence was found to be affected with the infection. During laboratory examination, 97/365(26.57%) of the *Staphyloccocus was isolated.* The relative proportional prevalence of *Staphylococcus* was 97/118(82.20%). They were found to be statistically significant (P<0.05). The highest mastitic dairy cows distribution were observed in megele 37(39.72%) while the lowest prevalence was seen in megele33 (26.02%) as indicated in Table 1. 22.87% and 39.2% of clinical and Subclinical mastitis prevalence were recorded . 13.72% and 35.84% of *S. aureus prevalence* were investigated in this study as Table 1 indicated. Consistently, of 365 milk samples examined from cows, 44(24.30%) and 74 (40.22%) , of the mastitis prevalence were seen in zebu and cross breeds, (P<0.001).

Table 1: Prevalence of mastitic Dairy cows in study sites

Study Sites	No of animals examined	Positive	Prevalence (%)	Chi2	p-value
Megele 37	73	29	39.72	2.61	0.62
Megele 29	73	23	31.50		
Amba 14	73	21	28.76		
Megele 33	73	19	26.02		
Megele 32	73	26	35.62		
Total	365	118	32.33		

Table 2: Prevalence of mastitic Dairy cows by CMT screening test in study sites

Study district	No of animals examined	CMT Positive (%)	Chi2	p-value
Abrahamo	365	118 (32.33%)	2.60	0.62

As pointing out in Table 2, the overall mastitis screened using CMT test were 32.3%; which was statistically non-significant(P>0.05) among the kebeles of the Abrahamo district.

Table 3: Prevalence of Staphylococcus spp

Study Sites	*No of animals examined*	*S.aureus (%)*	*Chi2*	*p-value*
Abrahamo	*365*	*97(26.57%)*	*276.55*	*0.000*

As shown in Table 3, the overall cow level *Staphylococcus (N*= 97/365 (26.57%) and it was significant(P< 0.05).

Table 4: Prevalence of mastitis types of clinical and sub clinical

Forms of mastitis	**No. of examined cows**	**No. +ve cows**	**No. *S.aureus* isolates**	**Prevalence of *S.aureus***	**X^2**	**P-value**
Clinical	153	35(22.87%)	21	21(13.72%)	276.55	0.000
Sub clinical	212	83(39.2%)	76	76(35.84%)		
Total	365	118(32.32%)	97	97(26.57%)		

(From the Table 4 point of view, 22.87% and 39.2% of clinical and Sub clinical mastitis was recorded and 13.72% and 35.84% of *S. aureus* was similarly driven in the study respectively).

Table 5: Prevalence of mastitis at breed level of Dairy cows

Breed	No of animal examined	No of positive (%)	X^2	p-value
Cross	184	74 (40.22%)	10.55	0.001
Local zebu	181	44(24.30%)		
Total	365	118 (32.3%)		

Of 365 milk samples examined from cows, Zebu (n=181, 44(24.30%), Cross (N=184; 74 (40.22%) cross breeds, under clinical examination and CMT screening test. The prevalence of mastitis among cross and zebu breeds has significant (P<0.001).

5.2. 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 6), the questionnaire survey and observation data result shows, age, breed, parity, pregnancy status, milking hygiene, pregnancy status, blind teat, teat lesion, body conditions, mastitis type, udder shape, and CMT reaction, are amongst the potential risk factors, which are associated with mastitis disease in dairy cows. Accordingly, mastitis prevalence showed significant variation among different teat lesion (0.002); breed(0.001), parity(0.000), age(0.001), blind teat groups (p = 0.007), udder shape (0.000), milking hygiene (p=0.000), mastitis type (0.000), and pregnancy status (p=0.000), body condition (0.000). However, previous mastitis history and treatment history , floor type, and stage of lactation, have no significant difference with mastitis (p>0.05).

Table 6: Result of multivariate logistic regression of attribute risk factors with mastitis

Factor	Categories	Total no examined	No (%) positives	Chi2	p-value
Age(years)	≤3 (year)	23	15 (65.2%)	13.74	0.001
	4-7 years	236	66 (27.96%)		
	> 7 years	106	37(34.90%)		
Breed	Cross	184	74(40.2%)	10.55	0.001
	Zebu	181	44(24.30%)		
Parity	1-3	201	89(44.27%)	30.56	0.000
	4-6	155	29(18.70%)		
	≥6	9	0(0%)		
Lactation Stage (m)	Early (≤3)	120	37(30.83%)	4.83	0.18
	Mid (4-6)	82	21(25.60%)		
	Late (7-9)	50	15(30%)		
	Dry (>9)	113	45(39.82%)		
Pregnancy Status	Pregnant	55	(0%)	30.93	0.000
	Non- Pregnant	310	118 (38.06%)		
Previous mastitis History	Infected	335	104 (31.04%)	3.07	0.08
	Non- infected	30	14(46.66%)		
Floor type	Concrete	149	46(30.87%)	0.24	0.62
	Muddy (soil)	216	72(33.33%)		
Milking hygiene	Good	225	37(16.44%)	67.65	0.000
	Poor	140	81(57.85%)		
Prevoius mastitis Rx history	Yes	37	14(37.84%)	0.57	0.45
	No	328	104(31.70%)		
Blind teat	No	292	104(35.61%)	7.21	0.007
	Yes	73	14(19.17%)		
Teat lesion	No	340	103(30.29%)	9.39	0.002
	Yes	25	15(60%)		
BCS	Good	185	29(15.67)	47.55	0.000
	poor	180	89(49.44)		
type of mastitis	Healthy	254	7(2.75)	333.88	0.000
	subclinical	81	81(100)		
	clinical	30	30(100)		
Udder shape	Pendiculous	76	53(69.73)	61.39	0.000

	High up	289	65(22.49)		

5.3. 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-7). 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%).

Table 7: 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

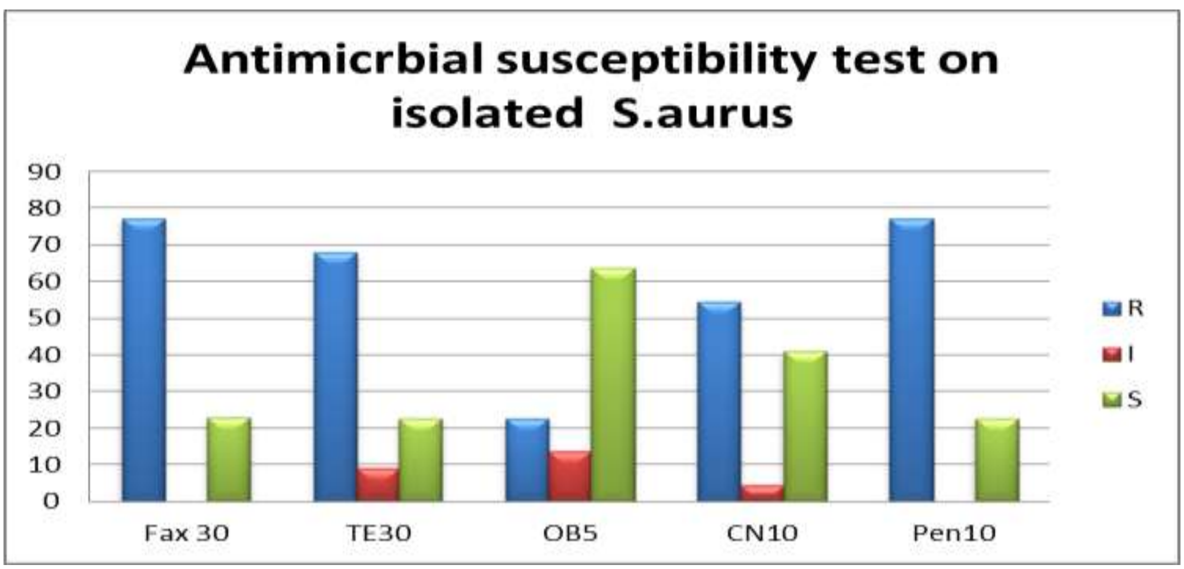

Figure 2: Antimicrobial drugs resistance and susceptibility profile of *S.aureus* isolated from milk
Key: *TE30=Tetracycline, Fax30= Cefoxitin, CN10=Gentamycin, Pen10= Penicillin G, OB5= Cloxacillin.*

6. DISCUSSION

In the present study, the overall prevalence of mastitic dairy cows were 32.3 % 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 prevalence of ***S.aureus*** were 97/365(26.57%). This finding is 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. Similarly, the present result was 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). 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, and Mekuria *et al*. (2013), 26.7%, 17.13%, 16.0%, 17.2%, 18.39%, 15.02%,, 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(24.30%) and cross breeds (40.2%) were significantly associated with the occurrence of mastitis ($P<0.001$). 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(30.83%) as compared to mid (25.60%) and late (30%) lactation stages was non- significant (p>0.05). The finding of higher infection in cows in early lactation stage followed by late and mid 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: concrete floor (30.87%) and muddy system (33.3%) of mastitis prevalence were identified, which has no significant influence on the occurrence of mastitis (p>0.05). 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=0.000). Cows at farms with poor milking hygiene standard are severely affected (57.85%) than those with good milking hygiene practices (16.44%). It was comparably with earlier reports of (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 significantly influenced by age categories (P <0.001), with the age less or equal to 3 year (65.2%); 4-7 years (27.96%) and greater than 7 years (34.90%) of infection rate. Similar result was reported by Shimelis (2014) in Selale /Fitche with significant effect. Besides this, it was in agreement with the report of Kerro and Tareke, (2003) who found that, the risk of clinical and sub clinical mastitis infection increases with the age of the cow. This might be due to the increased opportunity of infection with the time and the prolonged duration of infection, especially in herd without mastitis control program (Radostitis *et a*l., 2007).

In this study, parity is significantly influenced on the occurrence of mastitis (p<0.000). Comparably to this study, the increased occurrence of mastitis of cow parity was reported by Mekibib *et al*. (2010) in Holeta town and Haftu *et al*. (2012) in northern Ethiopia. The higher the parity number, the higher prevalence was observed in the studies of Tesfaye, (1995) and Biffa *et al*.(2005). According to Erskine (2001) report, primiparious cows have more effective defense mechanism than multiparious cows. But the present study showed a lower prevalence of mastitis at 3-4 births followed by in cows that gave 1-2, births and higher in cows that gave more than 5 births, so that, the occurrence of mastitis and parity revealed significant variation. This variation might be due to the influence of breed and management of the farm.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).

7. CONCULUSION AND RECOMMENDATIONS

Dairy cows mastitis could be one of the major constraints to dairy production in intensive dairy farms. Overall, 32.3% of mastitis and 26.57% of Staphylococcus prevalence were investigated in this research. Consistently, higher subclinical mastitis (39.2%) and lower (22.87%) clinical mastitis were reported. Similarly, 35.84% of subclinical staphylococcus and 13.72% of clinical staphylococcus were identified in this findings. Different potential risk factors were associated with mastitis in the study area, amongst these, age, breed, blind teat, teat lesion, milking hygiene, parity, body condition, previous mastitis, and pregnancy status of the dairy cows were prominent risk factors. 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;
- Hygiene measures during milking procedure should be practiced that may reduce the transmission of the disease,
- Staphylococcus prevention strategy should be initiated;
- Awareness should be created among dairy farm owners and dairy workers;
- Veterinarians should be aware of the risk factors;
- Risk factor controlling mechanism should be implemented
- During the selection and production of Dairy cows , teat and udder shape, infection / lesion on teat/, detection of Subclinical mastitis cases with animal health workers should be mandatory so as to gain productives cows,
- The risk factors like age, body conditions, blind teat, parity, previous mastitis history and housing status, pregnancy status etc will be assessed.

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8/5/2025