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Isolation and phenotypic characterization of *Streptococcus uberis* from mastitic cows in and around Batu town, Ethiopia.

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1 ABSTRACT

Streptococcus uberis **have emerged to be pathogens causing intramammary infections in dairy herds. In Ethiopia, knowledge about the *Streptococcus uberis* (*S.uberis*) involved in mastitis is limited. A cross-sectional study was carried out in Batu East shoa Zone of Oromia State, Ethiopia from December 2014 to April 2015 with the aim of isolation and phenotypic characterization of *Streptococcus uberis* from bovine mastitis. A total of 230 lactating (Holstein (n=115), Borena (68) and Jersey (n=71)) cows were included in this study and out of those, 97 (42.2%) were found to be affected with mastitis infection which was detected by clinical examination and the California Mastitis Test (CMT), of which 16(7%) and 81(35.2%) had clinical and subclinical mastitis, respectively. Positive milk samples were used for bacteriological examination and a total of 18 isolate of *S. uberis* were obtained. In the present study out of seven *in vitro antimicrobial* used, Nalidixic acid (37.5%), Amoxicillin (25%), Streptomycin (25%), Oxytetracycline (25%), Kanamycin (18.75%), Ceftriaxone (18.75%), and Compound sulphonamide (12.5%) showed resistance to *S. uberis*. Among the potential risk factors considered, age, parity, stage of lactation, breed and floor type were found to affect the occurrence of *S. uberis* mastitis significantly (P<0.05). In this study, it is observed that *S. uberis* should be given a great concern as a threat for the dairy industries.**

2 INTRODUCTION

Mastitis is inflammation of the parenchyma of the mammary gland regardless of the cause. Mastitis is therefore characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue. The most important changes in the milk include discoloration, the presence of clots and the presence of large numbers of leukocytes. There is swelling, heat, pain and oedema in the mammary gland in many clinical cases. However, the exact clinical and laboratory changes that occur in the udder because of infection can also be caused by other factors in the absence of infection (Radostits, 2006). *Staphylococcus aureus,* Coagulase Negative *Staphylococci* (CNS), *Streptococcus (Str.) dysgalactiae*, *Str. agalactiae* and *Str. uberis* are common causes of both clinical and subclinical mastitis (Pyorala, 1995). Coliform bacteria such as *Escherichia coli* and *Klebsiella* species most commonly cause clinical mastitis and seldom give rise to subclinical cases (Hogan and Smith, 2003). *Staphylococcus aureus* and *Str. agalactiae* are referred to as contagious udder pathogens as

they are bound to the bovine udder or the cow and are mainly transmitted from cow to cow. Good milking hygiene is one important factor in order not to spread these organisms within a herd (Pyorala, 1995). The coliform bacteria are called environmental pathogens as their main source of transmission is from the surroundings of the animal and are best managed by good environmental practices (Hogan and Smith, 2003) Coagulase negative *Staphylococci*, *Str. uberis* and *Str. dysgalactiae* are considered to be both contagious and environmental pathogens (Taponen and Pyorala, 2006). Identification of cows infected with mastitis is necessary to make decisions regarding treatment, culling or isolation of infected animals. Common methods used to identify infected cows include milk microbiology (cultures), the California Mastitis Test (CMT), individual cow Somatic cell count (SCC) values and electrical conductivity. Microbiologic exam of milk samples may be used for control programs (such as segregation plans) or for detection of new pathogens. Culturing is also used to determine antibiotic susceptibility of mastitis pathogens. Microbiologic examination of milk samples is often considered the standard for identification of infected quarters (Makovec and Ruegg, 2003). The genus *Streptococcus* consists of non-sporing, catalase negative facultative anaerobic bacteria. *Streptococci* have complex nutritional requirements and form mainly lactic acid or lactic, acetic and formic acids and ethanol and CO_2 from carbohydrates. *Streptococcus uberis* is commonly described as an environmental mastitis pathogen because it has an ability to survive and multiply in extramammary sites. It has been isolated from various anatomical sites of cows and from the cow's environment. These include bovine lips, skin, udder surface, belly, teats, urogenital tract, tonsil, rectum, rumen, nostrils, eyes and vulva. In addition, the organism can be isolated from water, soil, plant matter, bedding materials, flies, fecal samples and hay (Wongkattiya, 2008). The bacteria *Streptococcus uberis* (also known as *Strep uberis*) is a common cause of mastitis in dairy cattle in many countries around the world. *Streptococcus uberis* is found everywhere in the cow´s surroundings and mastitis often occurs in early lactation and in the end of the dry period (Pyorala, 1995). Symptoms are commonly moderate to severe (Milner *et al*., 1997). Environmental streptococci have become a major cause of mastitis in dairy cattle. Streptococcal infections are associated with many different species; however, the most prevalent species are *Streptococcus uberis* and *Streptococcus dysgalactiae*. Infections with these organisms can cause clinical mastitis that is commonly mild to moderate in nature (Radostits, 2006). *Streptococcus uberis* is classified within the order Lactobacillales and the family Streptococcaceae. It is belongs to the pyogenic group, displays either a weak α or γ-haemolysis, produces acids out of cellobiose, aesculin, glucose, fructose, galactose, inulin, maltose, mannitol, mannose, ribose, salicin, sorbitol, starch, sucrose and trehalose. *S. uberis* is able to adhere to and invade in mammary epithelia cells. Adherence and invasion can be attributed to the "*S.uberis* adhesion molecule". The high prevalence of *S. uberis* in some dairy herds may be explained by the ability to adhere to host cells. The enzymes of *S. uberis* seem to affect strongly the dissemination of infections caused by it. All strains produce free hyaluronidase that enhances the distribution of the pathogen within tissues. The hyaluronidase synthesized by *S. uberis* is capable of preventing the proliferation of a line of udder epithelial cells. Another factor of virulence could be its capability to produce hyaluronic acid capsules (Kromker *et al*., 2014). The Clinical Mastitis is accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008). The detection of clinical mastitis depends upon the examination of the mammary gland and its secretion. The affected gland may show swelling, heat, pain and hardness. The secretion may be clotted, serous or, occasionally bloodstained (Andrews *et al*., 2004). The type of

clinical mastitis can be classified into per acute (depression raised pulse and respiratory rates, loss of muscle coordination, cold extremities, reduced papillary reflex dehydration and diarrhea), acute (sudden onset, redness, swelling, hardness, pain, grossly abnormal milk and reduced milk yield. Systemic signs such as fever and lack of appetite and sub acute (minor alterations in the milk and the affected quarter such as clots, flakes or discoloured secretion. The quarter may also be slightly swollen and tender (Philpot and Nickerson, 2000). Subclinical mastitis is of great economic importance to dairy farmers because it results in reductions in milk yield and undesirable changes in the milk's composition (Brightling *et al.*, 2010), as well as increased costs associated with control strategies (Halasa *et al.*, 2009). It cannot be detected by visual observation, though it can be identified by conducting tests to detect the presence of infecting microorganism or the product of inflammation such as somatic cell (Philpot and Nickerson, 2000). Even though the study on *S.uberis* in lactating dairy cows was previously conducted in different parts of Ethiopia like Bishoftu town, in Areka town and Adama, but there is no study on the occurrence of *S.uberis* in the lactating cows in and around Batu town, where there are many high milk producing dairy farm. Therefore, the objectives of this study were:

- To determine the prevalence rate of *S.uberis* in mastitic cow in and around Batu town.
- To determine the associated risk factors for the prevalence of *S.uberis* in the study area.
- To test antibiotic susceptibility of *S.uberis*

3 METHODS AND MATERIALS

3.1 Study Area: The study was conducted in and around Batu from February 2015 to April 2015. Batu is located in the East Shoa Zone of the Oromia National state about 163 kilometres away from Addis Ababa. This town has a latitude of 7°9'N and 38°7'E longitude, with an elevation of 1650masl. The rainfall is bimodal unevenly distributed with an average annual rainfall of 761mm. It extends from February to September with a dry period in May to June. In winter there is much less rainfall than in summer. It has a tropical lowland climate. Monthly temperature variation is highly depend on rainfall, due to its location close to the equator and the seasons are only distinguished by the intensity of rain, which is the most in August and the least in December. The average annual temperature in Batu is 21 °C. The soil is fine sandy, loam with sand, silt clay in proportion of 34:48:18% respectively. Average PH of soil is 7.88 (CSA, 2010).

3.2 The Study Population and Sample Size Determination: The study animals include Holstein, Jersey and Borna (Zebu) breed lactating dairy cows managed in Batu town and its surrounding smallholder dairy farms. Dairy cows were kept as source of milk and yoghurt for the town and kept by larger dairy farms and smallholder farms. The average holding capacity per households was 6 but the range is from 2 to 22. All the cows in this study are hand milked and most of them milked two times a day during lactation period. The sample size was determined based on the formula given by Thrusfield (2007) considering 5% absolute precision, 95% level of significance and expected prevalence of 20%.

3.3 . Study Design: A cross- sectional study was carried out to determine the prevalence of *Streptococcus uberis* in bovine mastitis from December 2014 to April 2015 at cow and quarter level. Based on clinical manifestations for clinical mastitis and indirect test (California mastitis test and Culture) for sub clinical mastitis; Questionnaires and direct observations of the farms were used to collect information regarding the risk factors used in the analyses. Microbial isolation and *in-vitro* antibiotic susceptibility test using seven antimicrobial discs.

3.4. Data Collection: A questionnaire was developed and pretested, and all information relating to the study objectives was recorded. Data on each cow was collected in a format designed for this purpose. Relevant information was collected on cow history, housing system, milking practice, drug usage and other management practices. Risk factors considered were breed, age, parity, stage of lactation and floor type. Depending on clinical inspection and CMT results, cases were categorized as either positive or negative. Positive cases were further categorized as clinical and sub clinical mastitis.

3.5. Study Methodology

3.5.1. California Mastitis Test: Milk samples were collected from sub clinical mastitic cows and the samples were collected from each quarter and analyzed using CMT. From each quarters of udder, a squirt of milk sample was placed in each cup on the CMT paddle and an equal amount of 3% CMT reagent was added to each cup and mixed well (Quinn *et al*., 2004). When found positive, milk sample was further collected for bacteriological analysis from positive quarters and stored at 4°C for a maximum of 24 h until culturing.

3.5. 2. Milk Sample Collection, Transportation and Storage: After testing with CMT, the teat orifice of cows showing positive result was cleaned and disinfected using water, towel and cotton soaked in 70% ethyl alcohol and by following strict aseptic measures 4 to 5 ml of milk for bacteriology examination were collected by holding sterile universal bottle holding in slant position after discarding 2-3 streams of milk. The universal bottle were labelled, then put in ice containing icebox and transported immediately to the Addis Ababa University College of veterinary medicine and agriculture. The samples were stored at +4 °c for 24 hours (NMC, 2004).

3.5.3. Bacteriological procedure: Milk samples that had been refrigerated, dispersion of bacteria and fat were accomplished by warming the samples at room temperature (25°C) for about an hour and then mixed by shaking. The samples were allowed to stand for a while for the foam to disperse. One standard loop (0.01ml) of milk sample was streaked on 7% blood agar. The inoculated plate was incubated aerobically at 37° C. The plates were checked for growth after 24-48. For primary identification, colony size, shape, colour, haemolytic characteristics and Grams reaction. Since *Streptococcus uberis* is fastidious bacteria it does not grow on nutrient agar as other bacteria grown. Then, it was sub cultured to brain heart infusion agar.

Gram's staining: The suspected cultures of *Streptococcus species* were subjected to gram's stain and observed under a light microscope for gram's reaction, shape and cell arrangement. The gram stained smears from typical colonies that showed gram positive cocci occurring in long chain were taken as presumptive *Streptococcus uberis* (Quinn *et al*., 2004).

Catalase test: Pure culture of the isolates were picked using a sterile loop from the agar and mixed with a drop of 3% H_2O_2 on a clean glass slide. If the organism was catalase positive, bubbles of oxygen were liberated within a few seconds and catalase negative isolates did not produce bubbles. The catalase negative cocci were considered as *Streptococci* (Quinn, 2004) (Table 6).

Oxidation and fermentation test: Uninoculated media is green in colour and semisolid, when the bacteria was inoculated it change to yellow due production of acid by fermentation of glucose in the media. *Streptococcus* are fermentative bacteria, both sealed and open test tube were changed to yellow (Quinn, 2004).

Colony characteristic on Edward media (selective media for S.uberis): The colonies that were identified by gram staining and catalase test as *Streptococcus* were streaked on Edward media plates and incubated at 37°c and examined after 24-48 hours for growth and change in colour of the medium. The presences of growth, hemolysis and esculin hydrolysis (dark background) were one indication of *S. uberis* (Quinn *et al*., 2004).Then colonies that were grown on Edward media picked by a wire loop and

streaked on macConkey agar. The absences of growth on macConkey agar were an indication of *Streptococcus uberis* (Quinn *et al*., 2004) (Table 6).

3.6. Antibiotic Sensitivity Test: Susceptibility of bacteria to the commonly used antimicrobials was conducted using Kirby-Bauer method (Quinn *et al*., 2004). About seven antimicrobials such as, Sulphonamide (300µg), Nalidixic acid (30µg), St reptomycin (25µg), Amoxicillin (30µg), Ka-namycine (30µg), Oxytetracycline (30µg) and Ceftriaxone (30µg), were selected from main class of antimicrobials and investigated for sensitivity testing. The antibiotic disks were applied on the surface of the inoculated agar plates using aseptic technique. Each disk was pressed down to ensure complete contact with the agar surface. After measuring the zone of inhibition, it was classified as sensitive, intermediate and resistant according to National Committee for Clinical Laboratory Standard (NCCLS) break point to interpret the inhibition zone (CLSI, 2014).

3.7. Data Analysis: The data was entered to Microsoft Excel spreadsheet and analyzed by using SPSS version 20 computer software. The effect of risk factors such as age, breed, lactation stage, number of parity and farm hygiene with possible association of the disease was analyzed using chi- square. Values were considered significant at $P<0.05$ was considered significant in all analysis.

4 RESULTS

4.1. Physical Examination of Udder: The udder was checked by physical examination for the presence of swelling, pain, hotness, disproportional symmetry, fibrosis, visible injury, teat blindness and atrophy. It was also checked for the abnormalities of milk including flakes, clots and watery secretion.

4.2. Prevalence of Mastitis and *Streptococcus uberis*: A total of 230 Holstein, Jersey and Borena cows from farms and smallholders were examined for mastitis detection and out of which 97 (42.2%) cows were found to be affected with clinical and sub clinical mastitis based on the clinical diagnosis and CMT. Likewise, CMT positive for the sub clinical mastitis were found to be 81 (35.2%) and the clinical were 16(7%) (Figure 1). Out of the 920 quarters examined, 12 (1.304%) quarters that belongs to 12 (5.22%) animals were found to be blind teat (Table 1). Up on screening of the functional teats (920) by CMT, a quarter of 107 (11.63%) found to be affected by subclinical mastitis (Figure 1). The prevalence of isolate (*S. uberis*) in subclinical and clinical mastitis is 88.9 % (n=14) and 11.1 % (n=2) respectively (Figure 2). The overall prevalence of *S. uberis* is 7.8% and 10.17% at cow and quarter level, respectively (Table 2).

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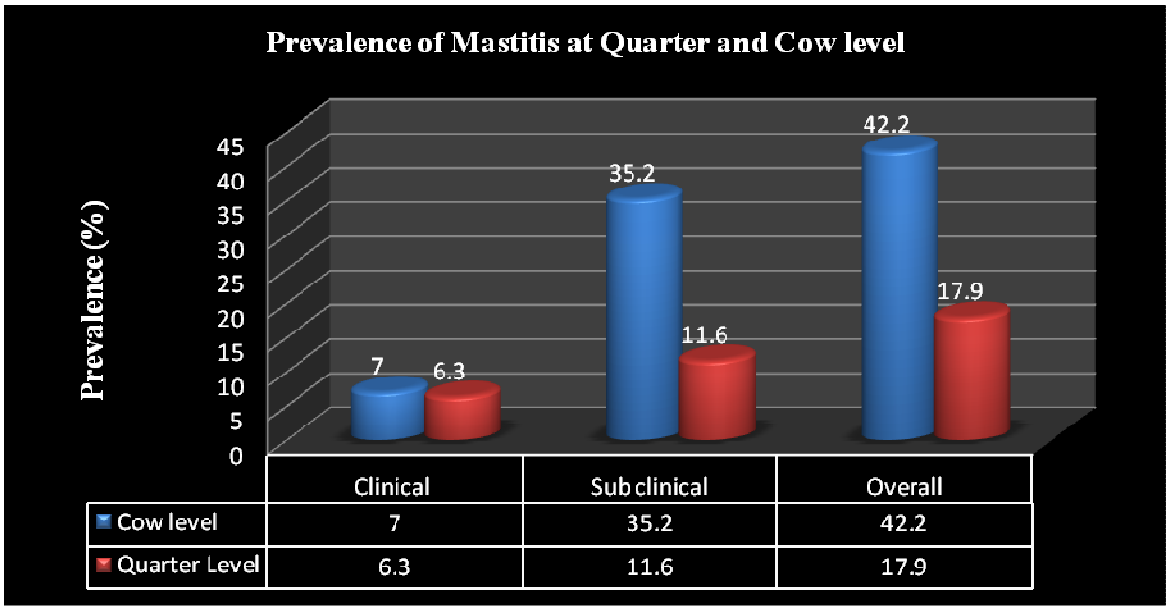

Figure 1: Prevalence of mastitis at cow and quarter level

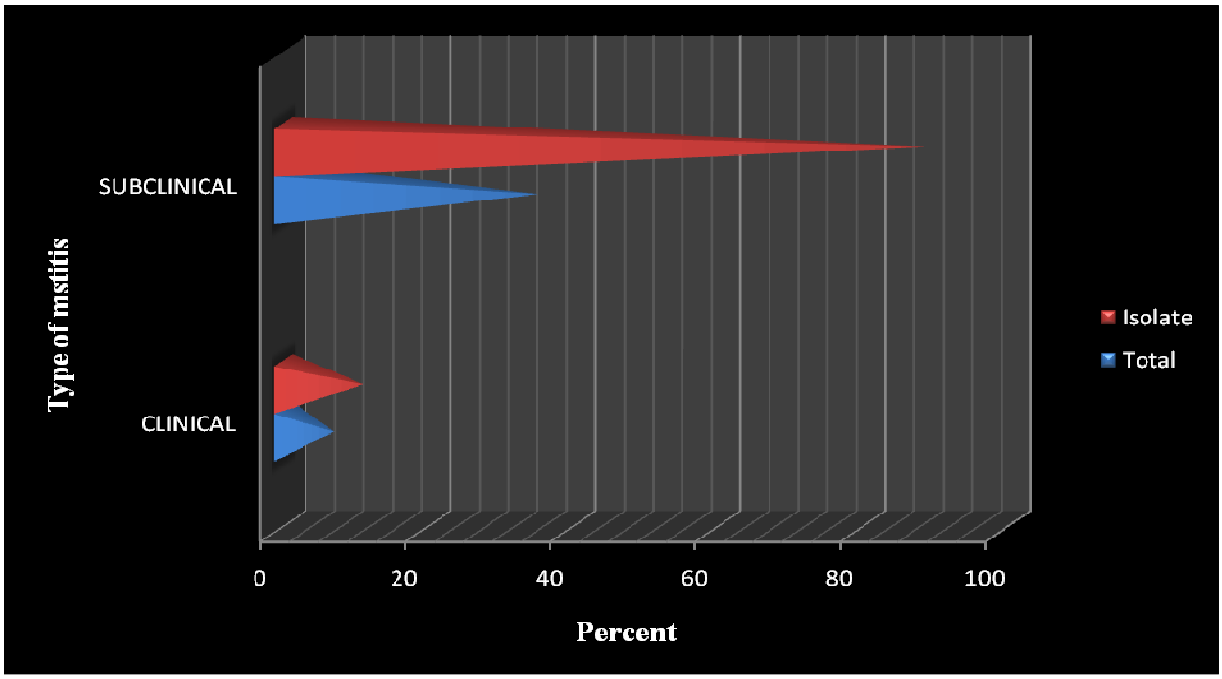

Figure 2: Prevalence of *S. uberis* isolates in clinical and subclinical mastitis at cow level.

Table 1: Quarter level prevalence of mastitis

Quarter	No examined	Blind	Negative	Positive	x^2	p-value

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RH	230	4(1.74%)	171(73.4%)	55(23.91%)	108.	.000
LH	230	6(2.61%)	172(74.78%)	52(22.61%)	106.3	.000
RF	230	1(0.435%)	193(83.91%)	36(15.65%)	60	.000
LF	230	1(0.435%)	195(84.78%)	34(14.78%)	56.6	.000
Total	920	12(1.304%)	731(79.46%)	177(19.24%)		

Key: RF=Right front; RH= right hind; LF= left front; LH= left hind

Table 2: The prevalence of *S. uberis* in the quarter level

Quarter	CMT positive	Culture for *S.uberis* n (%)
RH	55	7(3.0)
LH	52	6(2.6)
RF	36	3(1.5)
LF	34	2(0.8)

Key: n= number

4.3. Risk Factor Affecting the Prevalence of *S. uberis*: In this study, the risk factors of mastitis and *S. uberis* identified in Holstein, Jersey and Borena breed in farm and smallholder farming system were parity, age, breed, stage of lactation and hygienic conditions. Table 3 shows the results of measurement of association between mastitis and the risk factors. The result showed that the prevalence of mastitis was significantly higher (60.1) in animals older than 7 years followed by animals in the age range of 4 to 7 years and lowest in animals younger than 3 years. Parity also had effect on the occurrence of mastitis. The prevalence was higher in animals with higher number of births than those with fewer births (x^2 = 29.04, P = 0.000). It was followed by cows with parity of 3 to 4 and those with parity of 1 to 2 in that order. Statistically, highly significant association was observed between mastitis and hygienic status of the farms visited. The prevalence of mastitis was higher in cows on farms with poor hygiene than those on farms with good hygienic conditions (x^2 = 21.73, P = 0.000). Similarly, the stage of lactation was found to be associated with occurrence of mastitis in the area. The cows were at higher risk of acquiring mastitis when they were in their first 4 to 6 months of lactation and it was found to decrease as lactation stage decrease. **4.4. Biofilm Production Status:** A biofilm (slime-enclosed aggregates) is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance.

Congo red agar method: *Streptococcus uberis* strains were screened for their ability to produce slime by plating them on Congo red agar. A total of 16 pure isolated *S. uberis* were subjected for biofilm production on a modified Congo red agar. However, none of the isolate shows a black colony with dry crystalline consistency (0%), rather they were found forming a red colony, which indicates a bacterium with no biofilm production (Table 4).

Table 3: Risk factors associated with the prevalence of *S. uberis* cause of mastitis on dairy cattle.

Variable	Category	No.	Positive	Proportion	X^2	P-Value
Age	<4	99	22	22.2		
	4-7	60	32	53.33	29.07	.000

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	>7	71	43	60.53		
Parity no	Few (1-2)	99	22	22.2		
	Moderate (3-4)	59	31	52.54	29.035	.000
	Many (>4)	72	44	61.1		
Lactation stage(in month)	Early (1-3)	62	9	14.52		
	Mid (4-6)	93	49	52.69	26.63	.000
	Late (>6)	75	39	52		
Milk hygiene	Good	77	16	21.33		
	Poor	153	81	52.26	21.73	.000
Floor type	Concrete	136	42	30.88		
	Mud	94	54	58.51	17.4	.000
Breed	Holstein	115	61	53.04		
	Borena	71	23	32.39	11.2	.004
	Jersey	44	13	29.55		
Antibiotic use	Yes	97	19	19.59	3.675	.06
	No	133	41	30.83		

Table 4: *Streptococcus uberis* isolates and Biofilm production status

Number of isolated bacteria	**Biofilm production on MCRA**	
	Positive	**Negative**
16	0(0%)	16(100%)

MCRA: Modified Congo red agar: This might be due to milk components, PH of the milk; the environmental factors such as temperature, osmolality and PH influence the biofilm formation. Dipotassium hydrogen phosphate acts as buffering agent in milk. It protects the microorganisms from adverse environmental conditions like change in PH. A fall in the concentration of K_2HPO_4 reduces the buffering actions. Hence, it favours the bacteria for making irreversible attachment to the surface, the initial step for biofilm formation. Hydrogen ion concentration elicits significant a negative effect on biofilm formation (Atulya *et al.*, 2014).

4.5. Antimicrobial Susceptibility Profile: A total of 16 isolates from clinical and subclinical mastitis were tested for their *in vitro* antimicrobial sensitivity. The results showed that the majority of the isolates were highly sensitive to Compound sulphonamide, Oxytetracycline and Amoxicillin. *Streptococcus uberis* were highly resistant to Nalidixic acid (Table 5).

Table 5: Antimicrobial susceptibility test of *Streptococcus uberis*

Antibiotic disk	Susceptibility Test **S, n (%)**	**I, n (%)**	**R, n (%)**
Streptomycin	8(50)	4(25)	4(25)
Compound Sulphonamide	13(81.2)	1(6.25)	2(12.5)

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Oxytetracycline	12(75)	-	4(25)
Kanamycin	11(68.75)	2(12.5)	3(18.75)
Ceftriaxone	10(62.5)	3(18.75)	3(18.75)
Amoxicillin	12(75)	-	4(25)
Nalidixic acid	5(31.25)	5(31.25)	6(37.5)

Key: S= susceptible, R= resistant, n= number, -= no

4.6. Antimicrobial Resistance Patterns of *Streptococcus uberis* : From the total *S. uberis* isolate as shown in Figure 3 43.75%, where resistance to for one drug 18.75% for 2 drugs and 18.75% for 3 drugs.

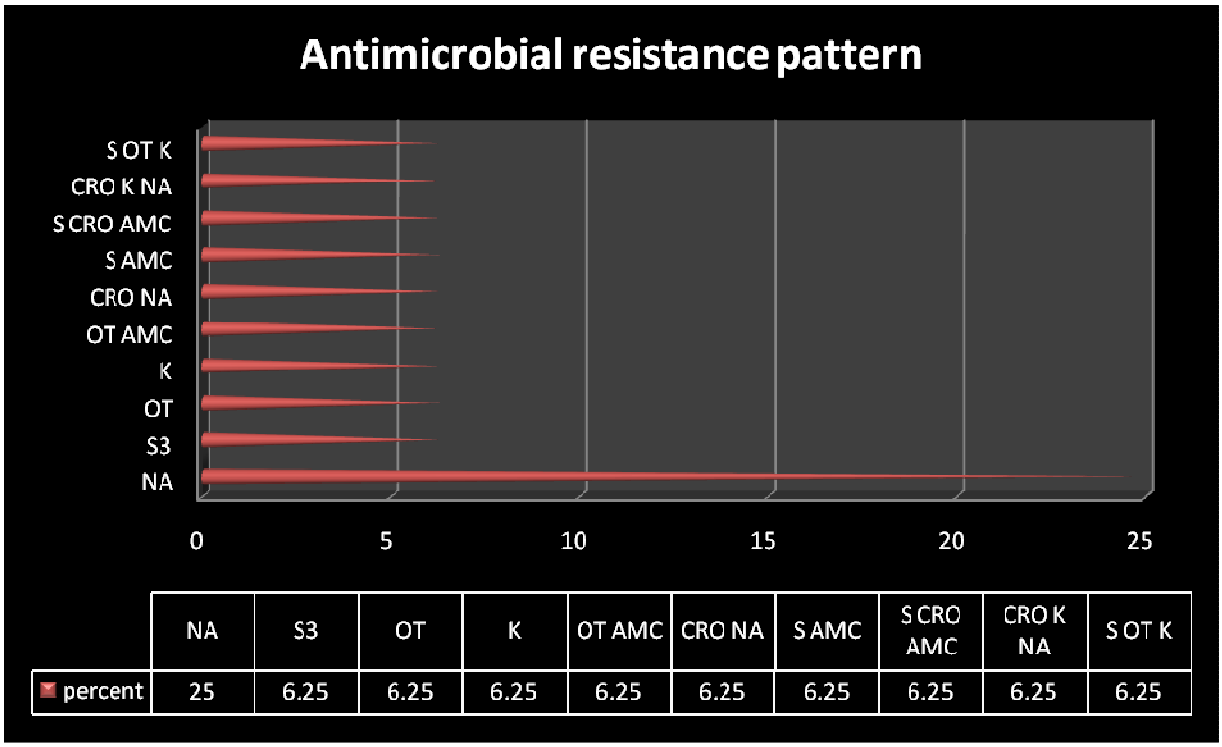

Figure 3: Number and proportion of resistant *Streptococcus uberis*

Table 6: Characteristic of *S. uberis* on media and biochemical test

No isolate	Edward media	MacConkey Agar	OF test	Antimicrobial Resistance pattern	Aesculin Hydrolysis	Hemolysis	Catalase test
1	+	-	F	NA	+	α	_
2	+	_	F	S CRO AMC	+	α	_
3	+	_	F	CRO K NA	+	α	_
4	+	_	F	OT AMC	+	α	_

5	+	-	F	S S3 AMC	+	α	_
6	+	_	F	NA	+	α	_
7	+	_	F	CRO NA	+	α	_
8	+	_	F	S3	+	α	_
9	+	_	F	OT	+	α	_
10	+	_	F	NA	+	α	_
11	+	_	F	AMC	+	α	_
12	+	_	F	S OT K	+	α	_
13	+	_	F	NA	+	α	_
14	+	_	F	S AMC	+	γ	_
15	+	_	F	K	+	γ	_
16	+	_	F	_	+	γ	_
17	+	_	F	_	+	α	_
18	+	_	F	_	+	α	_

Key: +=positive, - =negative, F= facultative or fermentative, α= alpha, γ= gamma, S= streptomycin, K=kanamycin, OT=oxtetracycline, CRO=, ceftriaxone, AMC=amoxicillin, S3=compound sulphonamide, NA= naldixic acid.

5 .DISCUSSION

In the current study, the overall prevalence of bovine mastitis is 42.2%. This prevalence is lower than those of Mekbib *et al.* (2010) and G/ Michael *et al.* (2012), who reported 71% in exotic dairy cows of Holeta farm and 52% Areka town respectively in elsewhere Ethiopia . This finding on the prevalence of mastitis are also lower than that of Rahman *et al.* (2010) and Salih *et al.* (2011) Benta and Habtamu (2011) who reported the prevalence of 53.30% in Bangladesh and 52% in Nigeria, 56.5% in Ethiopia respectively. The present study is higher than Girma *et al* (2011) and Tolosa *et al.* (2009) who reported 23.18%, and 27.3% respectively. This finding closely agrees with those of Darsema (1991) and Fekadu (1995) who reported, 39.8% and 38.65% respectively. This variation could be due to variation in the susceptibility of different breeds of cattle to mastitis causing organisms. The difference in management practices and environmental conditions could also be responsible for this variation. In addition, this study focused on the three breed, but others study considered one or two breeds. The present prevalence of subclinical mastitis is 35.0%, this result closely agree with Sorri *et al* (2005), Belayneh *et al.*(2009), and Abunna *et al.* (2013) who reported 36.0% ,33.6%, and 36.7% respectively. The prevalence of clinical mastitis is closely agree with Benta and Habtamu (2011), Girma *et al.* (2011), G/Michael *et al.* (2012) who reported 5.3%, 7.29% and 9.4% respectively. This finding of bovine subclinical mastitis is higher than that of Girma *et al.* (2011) and Tollosa *et al.* (2010) who reported 15.89% and 17.5% in Hararghe Doba district and Wolyta respectively. The clinical mastitis in this study is lower than Abunna *et al.* (2013) and Sorri *et al.* (2005) who reported 15.41% and 16.11% in different part of Ethiopia respectively. This variation could be hygienic condition and management. Among the risk factors considered, age , number of parity, stage of lactation, breed , farm hygiene and floor type found to be statistically significant ($P<0.05$). The occurrence of more cases of mastitis in older animals observed in the present study is in agreement with reports of Girma *et al* (2011), Abayneh *et al.* (2009) and Abunna *et al.* (2010). The previous investigation carried out elsewhere showed that the higher prevalence of mastitis in older animals is due to increased patency of teats and increased degree and frequency of previous exposure in multiparous old cows (Radostits, 2006). This investigation

also showed that prevalence of mastitis was lower in cows with fewer parities and the prevalence was higher in cows with multiple parities. This finding is disagree with G/ Michael *et al.* (2012). Several factors can be involved in the development of mastitis in animals with multiple parities. The risk of clinical and subclinical mastitis increases significantly with advanced age of cows, which approximates with parity number. This will increase the patency of the teats and decreases the local defence mechanisms. Repeated parturition also exposes cows to environmental and contagious bacteria. The immunity animals decrease through age making older animals more prone to mastitis. The significant difference among Holstein, Jersey and Borena breed may be associated with high milk yield and the structure of the udder is different in those breed. Holstein breeds have large udder, this may prone the udder to have close contact with the cow leg and the ground easily. This contact could create good opportunities for the environmental organism to enter in the udder and cause infection (Table 4). This finding on the significant of breed on mastitis is agree with Benta and Habtamu (2011) and G/Michael *et al.* (2012) who reported in Ethiopia (Batu and Areka town) on local and cross breed.

The relationship between the prevalence of mastitis on different lactation stage was studied; the result showed significantly higher infection ($p<0.05$) in cow with mid and late lactation than cow with early lactation stage. These results agree with Abayneh *et al.* (2009). The purpose of this study is not identifying the whole bacteria which cause mastitis, but it concentrate on *Streptococcus uberis*. Mostly *S.uberis* was isolated from subclinical mastitis as compared to clinical mastitis. The isolation prevalence of these bacteria was 7.8. This value closely agree with Girma *et al.* (2011), Abayneh *et al.* (2009), G/Michael *et al.* (2012) who reported 5.8% in Doba district, West Hararghe,6% in Adama town and 5.2% in Areka town, Southern Ethiopia respectively. However, it is higher than those of Siraj *et al* (2012), Abunna *et al* (2013) and Sori *et al.* (2005) who reported 3.06% in Ambo town, 4.23% in Addis Ababa and 2.99% in and around Sebeta respectively. In addition, the prevalence of *S.uberis* in this finding is higher than Mekonnen and Tesfaye (2010) and Idriss (2014), Sandra (2013) who reported 3.3% in Adama, 4.10% in Slovakia (in Nitra region) and 3% in Uganda respectively. The prevalence rate of *S.uberis* in this study is lower than Ayano *et al.* (2013) who reported 12.1% in Holeta, Ethiopia. This variation might be good herd management, teat dipping before and after milking, washing milkers hands before and after milking, preparation of clean towel for each lactating cow, milking of infected cow lastly, using dry cow therapy method and treating clinical cases at early stage. The prevalence of *S.uberis* in the current study in quarter level is 10.17%. This prevalence is higher than that of Guelat *et al.* (2014) who reported 1.3% in Switzerland. This might be difference in housing, house facilities (that predispose the accumulation of faeces on cows and bedding straw) and management practice, the breed of the cows. The *in vitro* antimicrobial susceptibility test showed that compound sulphonamide (81.25%) is the most effective drug followed by Oxytetracycline (75%) and Amoxicillin (75%) in the study area. Sensitivity of amoxicillin closely agrees with Girma *et al.* (2011) who isolate *Streptococcus uberis* in Doba district, West Hararghe. *Streptococcus uberis* showed resistance to Nalidixic acid. Susceptibility of Streptomycin was higher than Sumathi *et al.* (2008) who reported 35% in Bangalore. Streptomycin is resistance on *S.uberis* in Slovakia Idriss *et al.* (2014), but in this study the sensitivity of streptomycin is half percent or 50%, therefore Streptomycin is in this study sensitive than Idriss study, this might be streptomycin is extensively used drug for treatment of mastitis in Slovakia. The sensitivity of Streptomycin, Kanamycin and Amoxicillin are similar with Belayneh *et al.* (2009).

6 CONCLUSION AND RECOMMENDATIONS

Streptococcus uberis cause of mastitis is becoming a major health problem of dairy cows in the study area and undoubtedly will have an adverse effect on productivity of dairy industry. It is recognized that multiple environmental and managerial factors plays a major role in the occurrence of this pathogen. Furthermore, these groups of pathogens nowadays emerge as resistance to different antimicrobial agents especially for those commonly utilized in the study area. Hence, remains as a major agent for the occurrence of subclinical mastitis infection in the study area, which results in the economic loss of the dairy outputs.

Based on the above facts and the finding of present study the following recommendations are forwarded:

- Strict attention should be given for environmental pathogens and measures should be taken to control the hygienic status animal housing.
- Calves should be kept in clean area and dry cow therapy should be practiced.
- Mastitic cows needs to be milked last and there should be good habit of removal of dirty and slurry from the cows environment.
- Further research should be conducted to determine the prevalence and drug resistance features of S. uberis.
- Management and environmental factors should be regulated.

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