Research Article

Journal of Clinical, Medical, and Diagnostic Research

Bacterial Isolation and Identification from Bovine Mastitis and Their Pattern of Anti-Microbial Susceptibility Test in Selected Districts of North Shoa and Oromia Special Zone Surrounding Finfinne, Central Ethiopia

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Submitted: 2023, Nov 14; **Accepted:** 2023, Dec 18; **Published:** 2024, Jan 29

Citation: Belachew, T., Fufa, D. (2024). Bacterial Isolation and Identification from Bovine Mastitis and Their Pattern of Anti-Microbial Susceptibility Test in Selected Districts of North Shoa and Oromia Special Zone Surrounding Finfinne, Central Ethiopia. *J of Cli Med Dia Research, 2*(1), 01-09.

Abstract

A cross-sectional study was carried out from January 2023 to February 2023 to study the prevalence of bovine mastitis (Clinical and sub –clinical), risk factors, to isolate and identify major pathogens and to make antimicrobial susceptibility test on the isolates on cross breed lactating cows which kept under intensive farming /husbandry/ system found in Barek and Sululta town.

A total of 200 lactating cows examined 66 from Sululta, 71 from Wuchale and 63 from Debre libanos for mastitis by screening test/California Mastitis Test/ for sub clinical and udder observation and palpation for clinical mastitis from 9 kebeles which found in sululta, Wuchale and Debre libanos. The overall prevalence of mastitis was 57 (28.5%), where lower in Sululta and Debrelibanos districts 16(24.24) and 15(23.80) respectively and higher in Wuchale distirict 26(36.61) by clinical and sub clinical type of mastitis. From cultured samples, the bacteria genera species and isolated were as in with high prevalence were Staphylococcus intermidius 35(71.43), Staphylococcus aureus 6(12.25), E.coli 1(2.04). Coryne bacterium (6.12%) and Bacillus cereus 4(8.13). From currently tested few isolates using selected antimicrobial agents, all susceptible for Chloramphenicol and Gentamycin except one each isolates of S.aureus and for both which shows intermediate. Similarly, most of the isolates susceptible to Erythromycin and Amoxicillin except some isolates of Staphylococcus aureus, and bacillus cereus. However, high resistance was observed by most of isolates to Penicillin and tetracycline.

Those cows with different lactation stage and farming system revealed significant difference ($p<0.05$) in prevalence of mastitis. The prevalence of both age and parity by risk factors were insignificance ($P>0.05$), however, there was significance difference between semi intensive and extensive farming system ($P<0.05$). Prevalence of mastitis, particularly, the subclinical one could bring about major economic losses in dairy cows without notice as result of reduced milk production, poor growth or mortality of suckling calves and dam health. The resistance to penicillin and tetracycline were observed with poor efficacy may be due to prolonged and indiscriminate usage these antimicrobials in the area so that Chloramphenicol, Gentamycin and Erythromycin could be the drugs of choice in the present study.

Keywords: Mastitis, Prevalence, Risk factors, Antimicrobial

Abbreviations

CMT: California Mastitis Test
SCC: Somatic cell count
UK: United Kingdom
NMC: National Mastitis Council
NSAIDs: Non-steroidal anti-inflammatory agents

1. Introduction

Mastitis is a complex and multi factorial disease, the occurrence of which depends on variables related to the animal, environment and pathogen [1]. Among the pathogens, bacterial agent is the most common one, the greatest share of which resides widely distributed in the environment of dairy cows, hence a common threat to the mammary gland [2]. Mastitis induced via pathogenic microorganisms that generally come from two sources, either environmental

exposure of teat to contaminated environment, or the animal itself. *Staphylococcus aureus* and *Streptococcus agalactiae* which are predominant pathogens to cause bovine mastitis) that comprises contagious bacteria causing mastitis [3,1].

Mastitis can be classified as clinical or subclinical depending on the presence or absence of clear clinical signs. Clinical cases of mastitis are illustrated by the presence of one or more of symptoms such as abnormal milk, udder swelling and systemic signs including elevated temperature, lethargy and anorexia [3]. Mild clinical mastitis causes flakes or clots in the milk, whereas severe cases are associated with hot, swelling and discoloration of the udder, as well as abnormal secretion. Severe clinical mastitis can also exhibit systemic reactions, such as fever and loss of appetite. The duration of infection further classifies mastitis acute or chronic manifestations, where a sudden onset defines acute cases and an inflammatory process that lasts for months and results in progressive development of fibrous tissue characterizes chronic mastitis [4].

Mastitis can exist in the absence of visible signs of infection, and is then referred to as subclinical mastitis. It is the most prevalent form of mastitis [5]. Sub clinical mastitis are those in which no visible appearance of changes in the milk or udder, but milk production decreases, bacteria are present in the secretion and composition is altered. For every case of clinical mastitis there are 20-40 times as many cases of sub clinical mastitis [3]. The current standard method of detecting subclinical mastitis is to measure somatic cell count (SCC). Other inflammatory parameters, such as electrical conductivity, lactose, lactate dehydrogenase, acute phase proteins, etc., have been proposed as indicators of subclinical mastitis, and some have the potential of being adapted to in-line use [6]. Mild cases of clinical mastitis (abnormal secretion only)may not require treatment; however, all clinical mastitis episodes accompanied by an abnormal gland or systemic signs of illness should be treated with antimicrobial agents given by intra mammary infusion (all cases) and parenterally (selected cases). Acute and peracute mastitis cases require also supportive therapy (fluid and electrolytes) and non-steroidal anti-inflammatory a gents NSAIDs [7].

The cure rate after antimicrobial treatment of clinical mastitis is very variable due to both cow and bacterial factors such as parity of the cow, chronicity of the infection and bacterial genotype [8]. To approach appropriate treatment and control measure, it is important to perform antibiotic susceptibility test on relevant and most frequently used antimicrobials. Currently, in Ethiopia, including the study area, the information on prevalence and distribution of mastitis and the sensitivity to commonly used antimicrobials for treatment of mastitis is scarce.

Improvements of the productivity of livestock sector by controlling some of the major infectious disease, has received little attention in the country, especially, mastitis, the common problem of dairies that is known by an inflammation of the mammary gland is the leading one, that can contribute to reduce, milk production [9]. It is primarily resulting from an invasion of mammary tissues by pathogenic microorganisms through the teat canal resulting in physical, chemical, pathological changes in glandular tissues and milk. Evidence to date shows that affected dairy cows may loss 15% of their production and the affected quarter a 30% reduction in productivity [10]. There for the objective of the study was: Isolations and Identifications of pathogenic bacterial from bovine mastitis and their pattern of anti-microbial susceptibility test.

2. Materials and Methods

2.1. Study Area

Sululta District is among one of the six districts found in Oromia special zone surrounding Addis Ababa, Oromia Regional State. It lies between 390 30‘N Latitude and 380 30’ and 390 00 E longitude. It is located 40 km north west of Addis Ababa. The topography of Suluta is undulated with altitude ranging between 1600 and 3318m.a. s. l. The pattern of rainfall in the area is a bi-modal and ranges from 834 to 1440 mm. The short rainy season is between February and March while the long rainy season is between June and September. According to the Sululta Livestock Resource Development and Health office, the total cattle population of the district for the year 2011 was 210,211 heads.

Wuchale District is located at the 9º17’N-9º48’N latitude and 38º45’E-39º13’E longitude; 78 Km from Addis Ababa, the capital city of Ethiopia, and 34 Km from Fiche, the capital city of north shewa zone. The district has three major administrative towns as MukeTuri (district capital), Wobori, and Gimbichu. The highest elevation of the district is 2880m, the lowest is 1200 m, and the average elevation is 2412 m above sea level.

Debre Libanos District is located in 38o 58’ 33”E longitude and 9o 63’ 75”N latitude with altitude ranging from 1500 to 2700 m.a.s.l. For Debre Libanos the maximum and minimum annual temperature is 23 0C and 15 0C, respectively. Its main rainy season occurs between May and September and the dry season lasts from October to April. Clay and sandy soils are the major soil types of the districts. In the district agricultural production is characterized by a mixed crop-livestock production system [11].

2.2. Study Animals

The study populations were lactating cows breeds of cattle (Holstein Friesian (HF) and Cross breed (HF x Local) in selected dairy farms in the study area. The animals were managed under Intensive, semi intensive and extensive management system. They were often provided with some supplementary diet in addition to the natural pasture and agricultural by products and some were maintained usually in separate stalls, a short distance from each other in a house. Pre-milking and post-milking hygienic procedures, such as udder washing and drying, was frequently practice.

2.3. Study Design

A cross-sectional study was carried out from January to March to

study the prevalence of bovine mastitis (Clinical and sub –clinical), risk factors, to isolate and identify major pathogens and to make antimicrobial susceptibility test on the isolates on cross breed lactating cows which kept under intensive farming /husbandry/ system found in Sululta, Wuchale and Debre libanos Districts.

2.4. Sampling Methods and Determination of Sample Size

Simple random sampling was carried out and the sample size of the study was determined based on sample size determination method as described by with a 95% confidence interval and 5% desired precision [12]. The required sample size of the study animal was determined by the formula given in with 95% of confidence interval and 5% desired precision [12]. It is calculated by taking (89.54%) estimated prevalence from previous report by and 95% confidence levels and 5% precision level [13]. Simple random sampling method was employed to select the individual lactating cows.

$$N = \frac{(1.96)2 \times Pexp (1-Pexp)}{d^2}$$

whereas: - Pexp= Expected prevalence = 50%

d^2 d^2= Absolute precision = 5%

N = Sample size

Therefore, based on the above formula the sample size were 144 during the study period. Based on these 144 cows were expected to be sampled. However, to increase the precision in this study 200 lactating dairy cows were sampled from all study sites.

2.5. Study Methodology

2.5.1. Questionnaires' Interview

Data regarding the different potential risk factors (age, parity, lactation, district, house hygiene, udder hygiene, teat condition and use of towels was collected for 200 cross breed lactating cows by interviewing the animal owners/attendants. The classification of age (young < 5 years, adult 5-7 years and old ≥ 7 years), parity (few=1-3 calves, moderate=3-6 calves, many=≥6 calves) and lactation (early=1-4 months, mid=4-7 months, late= ≥ 7 months) were conducted based on [14,15,16] respectively.

2.5.2. California Mastitis Test (CMT)

Each selected lactating cow was screened for mastitis based on clinical examinations and California mastitis test. Clinical examination of the udder was based on the method indicated [7]. The clinical findings considered include abnormalities of the secretion, abnormalities of the udder and teat, and systemic reaction. The California mastitis test was performed according to established method [17]. About 2 ml 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 is applied in a horizontal plane for 15 s. 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 at least in one quarter ranging from Trace (T) to +3, based on gel formation. Hence, cow is considered mastitis positive, if one or more quarters were CMT positive.

2.5.2. Data and Sample Collection

A semi-structured and structured questionnaire was used to collect data on risk factors which include age, parity and hygiene of udder, farm owners and milker‟s. The milk sample which taken from mastitis positive cows not treated early with either intra mammary or systematic antimicrobials agents and collected according to protocol [18]. To explain: quarters was washed with tap water and dried. The teat ends are then cleaned with cotton soaked with 75% ethyl alcohol. Then, after discarding the first three streams of milk, 8-10 ml milk was collected aseptically into a sterile screw-cupped, pre-labeled test tube, by holding it in inclined position, so that, the pathogen that going to be recovered come from mammary gland. Finally, milk sample were held in an ice box for transportation to Asella regional veterinary laboratory for bacteriological examination to isolate mastitis causing bacteria and for antimicrobial sensitivity testing. The samples were immediately cultured or stored at 4 °C for a maximum of 24 h, until cultured on standard bacteriological media. Bacterium isolation and identification was carried out based on standard bacteriological techniques established [18]. In addition, data on potential risk factors including age, parity, lactation stage, housing and husbandry system are collecting from interview of owners and observation.

2.5.3. Bacteriological Milk Sample Examination

Culturing of milk sample collected from individual cows, in search for mastitis producing organisms in standard of examination for mastitis [7]. The milk samples were placed at room temperature if it is in refrigerator and were mixed gently. The bacteriological culture is formed following the standard microbiological technique and microbiological procedures for the diagnosis of bovine mastitis infection [19,20]. A loop full of milks streaked on 7% sheep blood agar plates are checked for growth after 24, 48 and up to 72 hours to rule out slow growing microorganisms such as Corynebacterium specie sample is considered negative if there is no growth after 48 hours or 72 hours. Samples are considered negative if there is profuse growth of environmental bacteria. For primary identification, colony size, shape color hemolytic characteristic, gram reactions are considered. These colonies are sub cultured to get pure colonies to nutrient agar, Mac Conkey agar, Edwards medium etc. other biochemical Estes. Characterizations of isolated mastitis causing bacteria was done by different methods, biochemical tests such as catalase, oxidase, coagulase, sugar fermentation test, oxidation fermentation test and indole test. The procedures adapted from for the identified pathogens are used [17].

2.5.4. Antibiotic Susceptibility Test

It was done by culturing the selected CPS and CNS isolates on Mueller Hinton agar media. The isolates were tested for 6 antimicrobials using the Kirby-Bauer disk diffusion method. The antimicrobial disks have various concentrations. Antibiotic discs were applied onto the inoculated MHA using disc dispenser and gently pressed to ensure intimate contact with the surface. The plates were

incubated aerobically at 37 °C for 18-24 h. The zones of inhibition were measured using a ruler and Oxford mathematical set divider. The results were reported as the diameter of the zone surrounding the individual disk in which bacterial growth was absent [21]. Based on this, the isolates were defined as resistant, intermediate and susceptible Antibiotic susceptibly tests were carried out on positive isolates and their susceptibility profile will be determined using Kirby-Baur disc diffusion method on Mueller-Hinton agar, following the procedures described by selected isolates from nutrient agar, are first cultured in to nutrient broth overnight [17]. A suspension of each test isolate is compared to a turbidity equivalent to a 0.5 McFarland standard and each suspension was streaked onto Mueller Hinton Agar by sterile swab. Then the following Oxoid antimicrobial susceptibility disks (Oxoid, Basing Stoke, and UK) with their corresponding concentration, Pencillin (P) (10ug), Ampicillin (AMP) (10ug), Chloramphenicol (C) (30 ug), Tetracycline (TE) (30 ug), Gentamicin (CN) (10 ug) and others antimicrobial disks were positioned on to the plates, using sterile forceps. Inoculated plates are incubated at 37 °C for 24 hr. The inhibition zone was recorded as the diameter of the zone surrounding the individual disc in which bacterial growth was absent. Based on this, the isolates are defined as resistant, intermediate and susceptible according to the guide lines of [17]

2.6. Data Management and Analysis

All the data obtained and collected during the course of study, risk factors, including: age, parity, lactation stage, housing and husbandry and the laboratory results are coded and entered in Microsoft excel data base system and subjected to descriptive statistics. The prevalence was calculated by dividing the number of positive animals for mastitis bacteria to the total number of mastitis positive animals examined times 100%. Prevalence rates of bacteria isolates was calculated as the number of times the bacteria species was identified over the total number of all the bacteria species identified. Resistance rates were calculated for each antibiotic and each bacterial isolate as the number of species isolates resistant over the total number of species tested. The overall resistance rates of each antibiotic were calculated as the number of bacteria resistant to antibiotic over total number of bacteria isolates tested [12].

3. Result

3.1. Prevalence

A total of 200 lactating cows examined 66 from Sululta, 71 from Wuchale and 63 from Debre libanos for mastitis by screening test/ California Mastitis Test/ for sub clinical and udder observation and palpation for clinical mastitis from 9 kebeles which found in sululta, Wuchale and Debre libanos (Table 1). The overall prevalence of mastitis was 57 (28.5%), where lower in Sululta and Debrelibanos districts 16(24.24) and 15(23.80) respectively and higher in Wuchale distirict 26(36.61) by clinical and sub clinical type of mastitis (Table 1).

Districts	Total number of samples	Positive (%)
Sululta	66	16(24.24)
Wuchale	71	26(36.61)
Debre libanos	63	15(23.80)
	200	57(28.5)

Table 1: Prevalence of bovine mastitis by districts

Out of 800 quarters examined during the study period 72(9.00%) were positive for mastitis while 13 (1.62%) blind (table 2).

Total Number of quarters examined	Positive	Percentage
800	72	9.00
Blind	13	1.62
Subclinical	67	8.40
Clinical	5	0.63

Table 2: Quarters level examined during the study period

Those cows with different lactation stage and farming system revealed significant difference ($p<0.05$) in prevalence of mastitis. The prevalence of both age and parity by risk factors were insignificance ($P>0.05$), however, there was significance difference between semi intensive and extensive farming system ($P<0.05$) as shown in table 3 below.

Risk factor	No of cow Examined	Positive No (%)	χ^2	p-value
Location stage Early Mid Late	91 64 45	24 (33.33) 20(27.77) 28 (38.88)	5.098	0.030
Age (yr) 3-6 6-9 9-13	112 52 36	27(37.50) 28(38.88) 17(23.61)	1.980	0.202
Parity 1-3 3-6 6-9	99 79 22	36(50) 19(26.38) 17(23.61)	1.423	0.070
Farm system Extensive Semi intensive	293 7	14(77.7) 4(22.2)	18.23	0.0100

Table 3: The prevalence of mastitis within the considered risk factors

From the test results in each districts S.epidermis higher in number and E.coli was lower than all isolate as shown in the table 4.

District	Total sample collected	Positive	negative	Bacteria name and no of sample test result
Sululta	15	10(66.7%)	5(33.3%)	S.epidermis(4),B.cereus(4) and Coryne bacteria(2)
Wachale	38	25(65.78%)	13(34.2%)	S.aures(4),S.epidermis(21),E.coli(1) and Coryne bacteria(1)
Derba libanos	19	12(63.16%)	7(36.84%)	S.aures(2) and S.epidermis(10)
	Total 72	37(51.38%)	25(34.72%)	

Table 4: Summary of test result by percentage and by districts

From cultured samples, the bacteria genera species and isolated were as in with high prevalence were Staphylococcus intermidius 35(71.43), Staphylococcus aureus 6(12.25), E.coli 1(2.04) Coryne bacterium (6.12%) and Bacillus cereus 4(8.13) as shown in table 5 below.

Isolates	Number of isolates
Staphylococcus aures	6(12.25)
Staphylococcus epidermis	35(71.43)
Escherichia coli(E.coli)	1(2.04)
Coryne bacterium	3(6.12)
Bacillus cereus	4(8.13)

Table 5: Overall prevalence of pathogen isolates causing bovine mastitis in the study area

Staphylococcus aures were highliy susceptibility (100%) to Oxacillin(OX), Kanamycin(K), Gentamicin(CN), Chloramphenicol(C), Ciprofloxacilin(Cip), Clindamycim(CD), Cefoxitin(FOX) and Vancomycin(VA) and resistant to PenicillinG(P), Amoxicillin(AX) and Ampicillin(AMP) as clearly explained in table 6.

			Staphylococcus aures (n=6)		
Class	**Antibiotics name**	**Potency**	**No. of susceptible strains (%)**	**No. of interme-diate-resistance strains (%)**	**No. of of resistant strains (%)**
β-Lactams	PenicillinG(P)	10unit	3(50%)	0	3(50%)
	Amoxicillin(AX)	2 µg	0	0	6(100%)
	Ampicillin(AMP)	10µg	3(50%)	0	3(50%)
	Oxacillin(OX)	1µg	6(100%)	0	0
Aminogycosides	Kanamycin(K)	30µg	6(100%)	0	0
	Gentamicin(CN)	10µg	6(100%)	0	0
	Streptomycin(S)	10µg	5(83.3%)	1(16.7%)	0
Tetracyclines	Tetracycline(T)	30µg	5(83.3%)		1(16.7%)
	Oxytetracy-cline(OT)	30µg	-	-	-
	Doxycycline(DXT)	30µg	4(66.7%)	0	2(33.3%)
Cephalosporins	Cefoxitin(FOX)	10µg	6(100%)	0	0
Macrolides	Erythromycin(E)	15 µg	5(83.3%)	0	1(16.7%)
Glycopeptides	Vancomycin(VA)	30µg	6(100%)	0	0
Salfonamides	Sulphamethax-azole-Trimetho-prim(SXT25µg)	23.75/ 1.25 µg	5(83.3%)	0	1(16.7%)
Phenicols	Chloramphenі-col(C)	30µg	6(100%)	0	0
Fluoroquinolones	Ciprofloxacilin(Cip)	5µg	6(100%)	0	0
Lincosamides	Clindamycim(CD)	2µg	6(100%)	0	0

Table 6: Summary of Resistances and susceptibility of Staphylococcus aures bacteria by percentage

Staphylococcus epidermis were Susceptible (100) to Kanamycin(K), Gentamicin(CN), Streptomycin(S), Cefoxitin(FOX), Vancomycin(VA), Sulphamethaxazole-Trimethoprim(SXT25) ,Chloramphenicol(C) ,Ciprofloxacilin(Cip) and Clindamycim(CD) and resistant (100) to Amoxicillin(AX) (Table 7).

			Staphylococcus epidermis (n=12)		
Class	**Antibiotics name**	**Potency**	**No. of susceptible strains (%)**	**No. of interme-diate-resistance strains (%)**	**No. of resistant strains (%)**
β-Lactams	PenicillinG(P)	10unit	5(41.7.2%)	0	7(58.3%)
	Amoxicillin(AX)	2 µg	0	0	12(100%)
	Ampicillin(AMP)	10µg	3(25%)	0	9(75%)
	Oxacillin(OX)	1µg	11(91.7%)	0	1(8.3%)
Aminogycosides	Kanamycin(K)	30µg	12(100%)	0	0
	Gentamicin(CN)	10µg	12(100%)	0	0
	Streptomycin(S)	10µg	12(100%)	0	0
Tetracyclines	Tetracycline(T)	30µg	6(50%)	0	6(50%)
	Oxytetracy-cline(OT)	30µg	-	-	-
	Doxycycline(DXT)	30µg	8(66.7%)	0	4(33.3%)

Cephalosporins	Cefoxitin(FOX)	10µg	12(100%)	0	0
Macrolides	Erythromycin(E)	15 µg	11(91.7%)	1(8.3%)	0
Glycopeptides	Vancomycin(VA)	30µg	12(100%)	0	0
Salfonamides	Sulphamethax-azole-Trimetho-prim(SXT25)	23.75/ 1.25 µg	12(100%)	0	0
Phenicols	Chloramphenicol(C)	30µg	12(100%)	0	0
Fluoroquinolones	Ciprofloxacil-in(Cip)	5µg	12(100%)	0	0
Lincosamides	Clindamycim(CD)	2µg	12(100%)	0	0

Table 7: Summary of Resistances and susceptibility of Staphylococcus epidermis bacteria by percentage

E.coli was resistant to Ampicillin (AMP) and almost susceptible the other drug used for the tests as shown in table 8 below.

			E.coli (N=1)		
Class	**Antibiotics name**	**Potency**	**No. of susceptible strains (%)**	**No. of interme-diate-resistance strains (%)**	**No. of resistant strains (%)**
β-Lactams	Ampicillin(AMP)	10 µg	0	0	1(100%)
Aminogycosides	Gentamicin(CN)	10 µg	1(100%)		
	Streptomycin(S)	10 µg	1(100%)		
Tetracyclines	Tetracycline(T)	30 µg	1(100%)		
	Oxytetracy-cline(OT)	30 µg	-	-	-
	Doxycycline(DXT)	30 µg	1(100%)		
Cephalosporins	Cefoxitin(FOX)	30µg	1(100%)		
	Ceftriaxone(CRO)	30 µg	1(100%)		
	Cefotaxime(CTX)	30 µg	1(100%)		
Fluoroquinolones	Ciprofloxacin(CiP)	5 µg	1(100%)		
β-Lactam combina-tion agent	Amoxicillin-clavu-lanate	20/10 µg	1(100%)		

Table 8: Summary of Resistances and susceptibility of E.coli bacteria by percentage

4. Discussion

The overall prevalence of mastitis in the present study was 28.50%, But this finding was lower than the 89.5% report of in salale, north shoa [13]. This could be due to difference in management system in that alternative free ranging management within door keeping system is mostly applied in the present study area. Environmental bacterial mastitis were higher in prevalence, due to poor housing facilities which predispose the accumulation of faeces on cows which will increase the rate of exposure of the teats and udder to the pathogens. Exposure to environmental Streptococci may occur during milking, between milking and during dry period [7].

From cultured samples, the bacteria genera species and isolated were as in (Table 4) with high prevalence were as in (Table 5) with high prevalence were Staphylococcus intermidius 35(71.43), Bacillus cereus 4(8.13), Staphylococcus aureus 6(12.25), Coryne bacterium (6.12%) and E.coli 1(2.04) from mastitis positive animal in present study shows the higher contributions of microbial in the cause of mastitis in the area.

Although Staphylococcus intermidius 35(71.43), this higher-level occurrence might be due to the organism's behavior which is adapted to survive in the udder and usually establishes a mild subclinical infection of long duration from which it shed in milk facilitating transmission to healthy animals mainly during milking and the predominant pathogens isolated in this study, it was lower than the 13% reported [7,13]. From cultured samples, the bacteria genera species and isolated were as in with high prevalence were Staphylococcus aureus (52.62%), E.coli (10.52%) Staphylococcus intermidius. Diplocuccus spps and Staphylococcus hycus (5.26%)

and Corynebacterium bovis (3.50%) were lower than the 38.4 and 23.2% report of irrespective of the agent [13].

From currently tested few isolates using selected antimicrobial agents, all susceptible for Chloramphenicol and Gentamycin except one each isolates of S.aureus and for both which shows intermediate. Similarly, most of the isolates susceptible to erytomycine except some isolates of Staphylococcus aureus, and bacilles cereus. However, high resistance was observed by most of isolates to Penicillin and tetracycline which is agree with finding of in and around Asella town [23].

5. Conclusion and Recommendations

The study attempted to prevalence of bovine mastitis by considering associated risk factors in north shoa zone, isolates and determines antibiotic susceptibility profiles of pathogens involved in bovine mastitis using clinical sing, CMT, bacteriology and invitro susceptibility test. Prevalence of mastitis, particularly, the subclinical one could bring about major economic losses in dairy cows without notice as result of reduced milk production, poor growth or mortality of suckling calves and dam health. Thus, due attention should be given to increase the awareness of health management (cow-udder hygiene, milking area hygiene, milker's hand hygiene, husbandry, tick control, etc.) in the area including proper antibiotic selection, dosage and frequency of administration of drugs.

Based on this concluding remark the following points can be recommended:

• Regular investigation of mastitis especially the subclinical form should be practiced.

• Good housing and udder/teats hygiene as well as appropriate treatment of cows during dry and lactation period should be practiced.

• Awareness should be created to dairy farm owners and dairy workers on the effect of mastitis.

• Mechanisms to control the risk factors associated with the disease should be implemented.

• Proper antibiotic selection, dosage and frequency of administration of drugs.

• A positive collaborative relationship has to be established between the regional, zone, district livestock development and health office, regional veterinary laboratory and owners so that they can be made aware of the impact of mastitis on their economy and public health risks and also what correction measures to be taken on it.

• Further study involving large area should be conducted on the role of pathogenic Staphylococcus species and susceptibility to microbial so as to undertake measurable control.

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