International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 1 Number 2 (2013) pp. 134-145

www.ijcrar.com

Study of bovine mastitis in asella government dairy farm of Oromia Regional state, South Eastern Ethiopia

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KEYWORDS

Mastitis;
Antibiotic susceptibility;
California mastitis test;
Asella;
Ethiopia.

A B S T R A C T

A study was conducted between December, 2012 and January, 2013 to estimate prevalence, isolate major bacterial pathogens for mastitis and to establish antimicrobial sensitivity for isolates in Asella government dairy farm. A total of 66 lactating cows and 264 quarters were constituted in the study based on clinical and California Mastitis Test (CMT). Accordingly, 44(66.6 %) cows had mastitis, which 8 (12.1%) clinical and 36(54.5 %) sub clinical and 111(42.04%) quarters were positive either clinically or under screening tests. Out of collected and cultured samples, 70(63%) samples were positive for aerobic and one facultative an aerobic bacteria. The following bacteria were isolated: *Staphylococcus aureus* (35.71%), Coagulase negative sthaphylococcus (15.71%), *Streptococcus spp* (11.42%), *staphylococcus intermidius* (7.14%), *E. coli* (5.71%), *P. haemolytica* (7.14%), *P.aureuginosa* (4.28%), *Bacillus species* (5.71%) and *micrococcus* species (7.14%). Out of eight *in-vitro* antimicrobials test, Gentamaycine, Chloroamphenicol and Kanamaycine were susceptible to all isolates and Vancomycine susceptible to gram positive isolates but many were resistance to penicillin and tetracyclines, which are most commonly, used drugs in the farm. Improving farm management system, regular screening test for sub clinical mastitis and use of antibiotics by sensitivity testing and culling chronically infected cows were recommended to reduce economic loss from

Introduction

Ethiopia is a country with a human population estimated to 85 million within annual population growth rate, 3.5% (CSA, 2010). Livestock represent a major national resource and form an integral part of the agricultural production system. In present day, there is a national drive to alleviate the existing food deficit by devising different agricultural strategies including 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 (Fekadu, 1995; Mekonnen *et al., 2005)*. 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 (Eriskine, 2001, Quinn *et al*., 2002; Radostitis, 2007). Evidence to date shows that affected dairy cows may loss 15% of their production and the affected quarter a 30% reduction in productivity (Heeschen, 1997).

Mastitis is usually classified as clinical and sub clinical based on aetiopathological findings and observation, clinical mastitis is further classified as per acute, chronic and gangrenous mastitis. It is most often sub clinical mastitis refers to inflammation of the mammary gland in the absence of visible gross lesion in the udder or it's secretion with the presence of pathogenic microorganisms and usually high number of somatic cells in the milk (Harmon, 1994; Radostitis *et al*., 2007).

The signs of inflammation such as swelling, heat, redness, gain or systemic responses, such as fever are not observed. Clinical mastitis refers to the condition where the cow's immune system responds with enough intensity to indicate signs of inflammation that is physically observable such as swelling, discoloration and pain (Radostitis *et al.,* 2007).

Efforts have only concentrated on the clinical cases, owing to heavy financial implications involved and the in evitable existence of latent infection, Mastitis is obviously an important factor that limits dairy production. The disease should be studied as it causes financial loss as a result of reduced milk yield, discarded milk following antibiotic therapy, Veterinary expense and culling of mastitic cows (Radostitis *et al*., 2007).

Majority of microorganisms that are responsible for mastitis and spoilage of milk could be *Staphylococcus aureus*, *Streptococcus agalactiae, Corynebacterium bovis*, *Mycoplasma* species, *Streptococcus uberis* (Erskine, 2001), coliforms (*Escherichia coli*, *Klebsiella* species and *Enterobacter aerogenes), Serratia, Pseudomonas, Proteus* species, environmental *Streptococci*, *Enterobacte*r species (Quinn *et al*., 2002). Besides many of them rendering milk and milk product unsuitable for human consumption, they are responsible for diseases like tuberculosis, streptococcal intoxication, colibacillosis, streptococcal sore throat and brucellosis in human (Radostits *et al*., 2007). As with most infectious disease, generally mastitis risk factors depend on three components; exposure to microbes, cow defense mechanism, environmental and management factors (Quinn *et al*., 2002).Besides improving herd health and dairy management. The controls of mastitis in dairy herds are accomplished in part with the aid of Antibiotics (NMC, 1999).Public hazards associated with the consumption of antibiotic contaminated milk results in allergic responses, changes in intestinal flora and development of antibiotic resistant pathogenic bacteria (Thirapatsakun, 1999). Because of animal health, its economic and public health importance, isolation and identification of the major bacterial agents and antimicrobial susceptibility test were important to reduce occurrence of drug resistance in the study farm.

Materials and Methods

Study Area

The present study was conducted in government dairy farm which is found in Asella town located in Oromia region, South Eastern Ethiopia. Asella town, the capital of Arsi zone, is located at about 175 km Southeast of Addis Ababa at 6° 59' to 8° 49' N latitudes and 38° 41' to 40° 44' E longitudes with an altitude of the area ranges from 2500 to 3000metre above sea level. Agricultural production system of the study area is of mixed crop and livestock production. Dairy farming using improved breeds is a common practice in urban and peri-urban areas (KARC, 2008).

The farming system is semi-intensive that run small to medium sized with up to 100 milking cows, most of which are cross-breed among Holstein-Friesian, Jersey and local Arsi breed introduced by the artificial insemination program and exotic breeds, since the establishment of CADU (Chilalo Agricultural Development Unit) in the mid-1960s by the Swedish- funded integrated rural development in Africa (Halderman, 2004).

Study population

The study population were 66 all lactating cows of the dairy enterprise which were cross breeds kept under semi intensive husbandry practice and there milking system was by hand (manual). Purposive sampling method was employed and relevant information about lactating cows in the farm was gathered. An attempt was made to examine all functional teats of each study animals for the isolation and identification of major mastitis causing bacteria as much as possible.

Study design and period

A cross- sectional study was carried out to determine bovine mastitis in December, 2012 and January, 2013 at cow and quarter level based on clinical manifestations for clinical mastitis and indirect test (California mastitis test and Culture) for sub clinical mastitis; microbial isolation and *in-vitro* antibiotic susceptibility test using eight antimicrobial disc diffusion.

Study methodology

Farm Inspection

The dairy farm was inspected for cleanness and other factors associated with mastitis and its bacterial isolation.

Data collection

Data on each sampled cow were collected in properly designed format.

Clinical inspection of the udder

The udder was examined clinically, using visual, then through palpation to detect possible swelling, pain, and disproportional symmetry, blindness of teats and discoloration of milk for the presence of mastitis according to (Quinn *et al.*, 2002).

Detection of mastitis

The Californian mastitis reagent was used to screen cows with sub clinical mastitis milk sample collection was according to the procedures recommended by national mastitis council (NMC, 1999). The result of the test was indicated on the basis of gel formation. The interpretation (grades) of the CMT was evocated and the results graded as 0 for negative and trace 1, 2 and 3, for positive (Quinn *et al.*, 2002).

Microbial investigation of mastitis

Milk Sample Collection

The milk sample was taken from cows not treated early with either intra mammary or systematic antimicrobials agents. For good collection of sample the teat were wiped thoroughly with 75% ethyl alcohol. The sterile collection of bottle was used and the first stream of milk from each quarter was discarded. The milk sample then held in an ice box for transportation to the laboratory. In laboratory samples was cultured immediately or stored at $+4^0$C (NMC, 1999).

Methods of Transportation and storage of samples

After collection of the milk sample, all samples were clearly labeled with the appropriate identification of the cows identification number, quarter using permanent marker on the test tube and all samples were transported with ice box to the laboratory without delay and it were processed immediately (Quinn *et al*., 2002). In the laboratory, samples were cultured immediately or stored at $+4^0$c in any case of delay (NMC, 1999). Analysis of specified samples was performed on isolation and identification of pathogenic bacteria at Asella regional veterinary laboratory in microbiology section.

Direct microscopy

The milk sample was centrifuged and stained smear made from the deposit. A Gram stain was used routinely. The Zehil Neelson staining was performed for rare cases when bacteria such as *M. bovis* are suspected (Quinn *et al*., 2002).

Preparation of culture media

To prepare media for bacterial culture, the manufacturer's instructions should be followed, besides few additional general points were included, all glass wares used for the preparation of media were first sterilized using appropriate equipment like autoclave, hot air oven, the appropriate amount of dehydrated media were weighed out of using sensitive balance and the required amount of distilled water were added to the powder media. Dehydrated media containing agar were dissolved in heating mantle until it boil and frothy appearance was settled(removed), then the media were sterilized by autoclave at 121^0C for 15 min holding time, and cooled in water bath at 50^0C before poured in to the Petri dishes. Some media like blood agar requires addition of blood after it is cooled to 50^0C since RBC are not tolerate higher temperature, adapted from Quinn et al. (2002). The common media used during the study were blood agar, MacConkey agar (Oxiod Hampshire, England), manitol salt agar (Oxiod Hampshire, England), Edward medium (Oxiod Hampshire, England) Eosin metheylne blue medium (Oxiod, Hampshire, England) and Triple sugar iron agar (Merck Germany) and Simon citrate of medium (BBL, Becton Dickinson), Trypton Soya broth (Oxiod Hampshire, England) and MR-VP biochemical media were used.

Culture

Before milking, milk samples were collected aseptically for microbiological culture, according to the procedures of the National Mastitis Council, 1999.Culturing of milk sample collected from individual cows, in search for mastitis producing organisms in standard of examination for mastitis (Radostits *et al*., 2007).

Biochemical tests

For the primary isolation and identification of mastitis causing micro organisms,

colony size, Shape, color, pigmentation, hemolytic characteristic, Gram's reaction, Oxidase, O-F tests were performed. After these colonies were sub cultured to different media, such as Manitol salt agar, MacConkey agar (Oxiod, Hampshire, England), Edward's medium (Oxiod Hampshire, England), Eosin metheylene blue medium (EMB) (Oxiod, Hampshire, England), etc to get a pure culture. And the secondary biochemical tests such as, coagulase test, urease test IMVIC tests, sugar tests, etc were done for bacterial species identification. The procedures for the identified pathogens were referred from Quinn *et al*. (2002).

Antimicrobial sensitivity testing

Susceptibility of bacteria to the commonly used antimicrobials was conducted using Kirby-Bauer method (Quinn *et al*., 2002). About eight antimicrobials such as chloroamphenicol, Gentamycine, Penicillin, Sulphamethizole, Streptomycin, Kanamycine, Tetracycline and Vancomycine (Oxiod, Hampshire, England) 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 (Quinn *et al.,* 2002).

Data management and analysis

Descriptive statistics were used to summarize the generated data on the rate which is collected through, clinical inspection, CMT, pathogenic bacteria isolation and identification and antimicrobial sensitivity test result were enter into data base management soft ware Microsoft Excel computer program 2003. The prevalence was expressed using percentage.

Result and Discussion

Prevalence of clinical and subclinical mastitis at cow level

A total of 66 lactating cows were examined, 8 (12.1 %) and 36(54%) were positive for clinical and sub clinical mastitis respectively. The over all mastitis prevalence in the farm were 44 (66.6 %) using CMT screening test (table 1).

Prevalence of mastitis at quarter level

A total of 264 quarter were examined, of which 111 (42%) were positive for mastitis while 25(9.4%) were blind with similar proportion of mastitis at each quarter (table 2).

Prevalence of mastitis at cow and quarter level

The prevalence of clinical and sub clinical mastitis at cow and quarter level were 8(12.1%), 36(54.5 %) and 27(10%), 84(32%) respectively (table 3).

Bacterial isolation

A total of 111 quarters were positive either clinically or under screening test using California Mastitis Test. All positive

Table.1 Prevalence of clinical and subclinical mastitis at cow level

Mastitis condition	Total number of examined	Result (%)
Clinical	66	8(12.1)
Subclinical	66	36(54.5)
Total	66	44(66.6)

Table.2 Quarter level prevalence of mastitis

Quarter	Number of examined	Blind quarter	Negative quarter	Positive quarter
Right back	66	15(22.7)	38(57.5)	32(48)
Left back	66	4(1.5)	39(59.0)	37(56)
Right front	66	4(1.5)	25(37.8)	27(40.9)
Left front	66	2(3.3)	26(39.3)	15(22.7)
Over all	264	25(9.4)	128(48.48)	111(42)

Table.3 Prevalence of mastitis at cow and quarter level

	Prevalence n (%)		
	Clinical mastitis	Sub clinical mastitis	overall
Cow level	8(12.1)	36 (54.5)	44(66.6)
Quarter level	27(10)	84(32)	111(42)

Table.4 Frequency distribution of bacterial isolates from mastitic cows

Bacterial isolates	Frequency	%
Staphylococcus aureus	25	35.71
CNS	11	15.71
Streptococcus species	8	11.43
Staphylococcus intermidius	5	7.14
E .coli	4	5.71
P. haemolytica	5	7.14
P. aureuginosa	3	4.29
Bacillius species	4	5.71
Micrococcus species	5	7.14
Total	70	100

samples were cultured; 70(63.06%) were positive for aerobic and one facultative an aerobic bacteria. The following bacteria were isolated with high prevalence of *Staphylococcus aureus* (35.71%), Coagulase CNS (15.71*%), Streptococcus spp (*11.42*%), Staphylococcus intermidius (*7.14%), *E. coli* (5.71%), *P. haemolytical* (7.14%), P.aureuginosa (4.28%), *Bacillus species* (5.71%) and *micrococcus* species (7.14%).

Antimicrobial Susceptibility profile

Of total positive samples, 29 were tested for susceptibility to different eight antimicrobial discs. The comparative efficacies of antimicrobials used indicates Gentamycine, Chloroamphenicol ,Kanamycine and Vancomycine were the most effective antibiotics where by 93.1%, 75.8%, 58.6% and 72.4%, respectively. Vancomycine was sensitive for all gram positive and resistance for all gram negative. Tetracycline, Sulfamethizole, penicillin and Streptomycin were showed very poor efficacies on many isolates, where by only 20.6%, 34.4%, 51.7% and 44.8% respectively.

In the current study *staphylococcus aureus, staphylococcus intermidius, Coagulase negative staphylococcus and micrococcus spp* isolates were more sensitive to Gentamycine (100%), Chloroamphencole (100%), and Vancomycine (100%). Similarly *Bacillus species* isolates were more sensitive to Vancomycine (100%) and penicillin (100%) and resistance to Tetracycline and Gentamicin with the isolates having 0% and 60% sensitivity respectively. *P.aerouginosa* isolates were more sensitive to Penicillin (100%), Gentamicin (100%) and Streptomycin (100%) and resistance to Tetracycline, Sulphamethizole and Kanamycine with the isolates having 100% 0% and50 % Sensitivity, respectively. Whereas *P.haemolytica* isolates were more sensitive to tetracycline (100 %), Gentamycine (100%) and streptomycin (100%) and resistance to Vancomycine, Penicillin, Sulfamethizole and Kanamycine with the isolates having sensitivity of 0% and 50%, respectively. *E .coli* isolates were more sensitive to Gentamycine (100%) and streptomycin (100%) and resistance to Vancamycine, Penicillin, Kanamycine and Sulfahamethizole with the isolates having sensitivity of 0% and 50%, respectively.

This study showed the overall prevalence of mastitis in crossbreed cows in Asella government dairy farm to be (66.6 %), which is in agreement with the reports on bovine mastitis reported by (Bedada and Hiko 2011) (66.1%) ; (Lakew *et al*.,2009) (65.6%) in and around Asella, Ethiopia. However, the prevalence reported in this study is lower than the previous report of (68.1%) by (Zerihun 1996) in directly related with the variation in the Addis Ababa, (Makibib *et al.*, 2010*) (*71.0%) in Holeta and (85.6%) by (Nesru 1986) in Dire-Dawa. On the other hand, the report of (Biffa1994) (33.0%) was lower than the present findings, and the reports of (Biru1989) (63.4%); (Tola 1996) (61.11%). The variability in the prevalence of bovine mastitis between reports could be attributed to difference in management of the farms, breeds considered, or technical know-how of the investigators.

The prevalence 12.1% clinical and 54.5% sub clinical type of mastitis positive were comparable with that of (Bedada and Hiko 2011) who reported 10.3% and 55.8% for clinical and sub clinical mastitis respectively. The prevalence of clinical mastitis lower 21.5% which reported by Workineh *et al.*, (2002) in Ethiopia and the

Table.5 Antibiotic Sensitivity Testing (n = 29)

Antibiotics	Unit	Number of organisms susceptible to different antimicrobial agents n (%)																												N (%)
		S.aureus			*S.intermidius*			CNS			Micrococcus sps			Streptococcus			Bacillus sps			*P. aureuginosa*			P. heamolytica			E. coli			
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	
CN	10 µg	6 (100)	-	0	4 (100)	-	0	2 (100)	-	0	2 (100)	-	0	3 (60)	-	2 (40)	2 (100)	-	0	2 (100)	-	0	2 (100)	-	0	4 (100)	-	-	93.1
C	30 µg	6 (100)	-	0	4 (100)	-	0	2 (100)	-	0	2 (100)	-	0	4 (80)	-	1 (20)	1 (50)	-	1 (50)	0	-	2 (100)	1 (50)	-	1 (50)	2 (50)	-	2 (50)	75.8
VA	30 µg	6 (100)	-	0	4 (100)	-	0	2 (100)	-	0	2 (100)	-	0	5 (100)	-	0	2 (100)	-	0	0	-	2 (100)	0	-	2 (100)	0	-	4 (100)	72.4
K	30 µg	6 (100)	-	0	4 (100)	-	0	1 (50)	-	1 (50)	1(50)	-	1 (50)	1 (20)	-	4 (80)	1 (50)	-	1 (50)	1 (50)	-	1 (50)	1 (50)	-	1 (50)	2 (50)	-	2(50)	58.6
P	10 µg	0	-	6 (100)	2(50)	-	2 (50)	2 (100)	-	0	1(50)	-	1 (50)	5 (100)	-	0	2 (100)	-	0	2 (100)	-	0	1 (50)	-	1 (50)	0	-	4 (100)	51.7
S	10 µg	0	-	6 (100)	2(50)	-	2 (50)	1(50)	-	1 (50)	1(50)	-	1 (50)	0	-	5 (100)	1 (50)	-	0	2 (100)	-	0	2 (100)	-	0	4 (100)	-	0	44.8
U	10 µg	0	-	6 (100)	2(50)	-	2 (50)	1(50)	-	1 (50)	1(50)	-	1 (50)	0	-	5 (100)	2 (100)	-	0	1 (50)	-	1 (50)	1 (50)	-	1 (50)	2 (50)	-	2 (50)	34.4
TE	30 µg	0	-	6 (100)	1(25)	-	3 (75)	1(50)	-	1 (50)	0	-	2 (100)	0	-	5 (100)	1 (50)	-	1 (50)	0	-	2 (100)	2 (100)	-	0	1 (25)	-	3 (75)	20.6

Keys: N = Number of observation, S = Susceptible, I = Intermediate, R = Resistance, C = Chloramphenicol, CN = Gentamycin, TE = Tetracycline, P = Penicillin, VA = Vancomycin, S = Streptomycin, U = Sulphamethizole, K = Kanamaycin

sub clincal finding was lower than the 89.5% subclinical mastitis report of Argaw and Tolosa (2008). This could be due to difference in management system in that alternative free ranging management with indoor 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 (Radostits *et al.*, 2007).

Isolation and identification of pathogenic bacteria such *Staphylococcus aureus, , CNS, Staphylococcus intermidius , Micrococcus* species ,*Streptococcus* species , *Bacillius species, P. hemolytica, P. aureuginosa, E .coli* at a rate of 63.06% from mastitis positive animal in present study shows the higher contributions of microbial in the cause of mastitis in the farm. Although *S. aureus* (35.71%) were similar with the 39.1% report of (Bedada and Hiko 2011) and the predominant pathogens isolated in this study, it was lower than the 47.1 % reported (Makibib *et al.*, 2010). But the present findings, coagulated by negative *staphylococcus* (CNS) (15.71%) and *S. intermedius (*7.14%), were lower than the (38.4%) and 23.2% report of Argaw and Tolosa (2008) irrespective of the agent. According to Pyorala and Vesa (1995) over 30% sub clinical and nearly 20% of acute cases of mastitis were usually due to CNS which is in agreement with the present result. The recent CNS isolated from bovine and other dairy animals mastitis milk samples (Ameh *et al.*, 1995) indicates that they could be pathogenic and may even cause more mastitis than *S. aureus.* This and such reports of *Staphylococcus* infection might be due to the fact that they are easily transmitted during milking via the teat cups and milker's hands. The primary reservoir of contagious pathogens includes *S. aureus* infected quarter.

The 7.14% isolated *Micrococcus* species in this study was comparable with the finding of (Bedada and Hiko 2011) and Mekonnen et al. (2005) who reported 5.6 and 10.2%, respectively. But it was much lower than that of Ameni et al. (2003) who reported 26.67% in different part of Ethiopia. Such differences and similarities may result from management system and ecological difference in agent. Similar reasoning was given by Ameni *et al.* (2003) and Mekonnen *et al.*, (2005).

The 5.71% isolation rate of *E. coli* found in this study was comparable with the findings of (Makibib *et al.*, 2010) who reported 4.6%. The prevalence of environmental *E. coli* may be associated with poor farm cleanliness and poor slope of stable areas. Faeces which are common sources of *E. coli* can contaminate the premium directly or indirectly through bedding, calving stalls, udder wash water and milker's hands (Radostits *et al.*, 2007).

The 5.71% *Bacillus* species isolated were similar with 6.52% finding of Belay (2008). It may be responsible for mastitis. (Radostits *et al.*, 2007) reported that *Bacillus* species only occasionally mastitis causing pathogens. The infection is associated with contamination of teat injures and surgery. The level of infection can be high during the dry period following the use of dry cow therapy preparation which may have been contaminated with the organisms.

When the overall result of antimicrobials susceptibility test in the present study was

compared on all isolates, Gentamaycine, Chloroamphenicol and Kanamaycine were the most effective antibiotics as 80 to 100% of the total isolates were found to be susceptible. Because these drugs were the least frequently used in the study area in Veterinary services. Thus no more resistance was developed. Similar suggestion was given by Jaims *et al.,* (2002) in that the development of antibiotic resistance is nearly always as a result of repeated therapeutic use and/or indiscriminate usage them. The present bacterial isolation from eight mastitis positive animal with previous using penicillin could be due to development of resistance to penstrep which was the common drug used for mastitis treatment in the study farm. Moreover, most of the isolates were penicillin resistance in present study. In this study *S. aureus* isolates were most susceptible to chloramphenicol, gentamycin and kanamycin while resistance to tetracycline and penicillin could be due to frequent usage of the latter two drugs in animal health. Similarly *E. coli* was highly resistant to penicillin and streptomycin but highly susceptible to chloramphenicol due to its infrequent usage. These were comparable with the findings of Mekonnen *et al.*, (2005).

The study attempted to investigate bovine mastitis using farm inspection, California mastitis test (CMT), bacterial isolation and invitro antimicrobial susceptibility test in cross breeds of lactating cows in the farm. The sub clinical mastitis was more common and important when compared to clinical mastitis. This may be due to the fact that farm managements and even veterinarian give more attention to clinical mastitis. The major isolates were contagious pathogens such as *staphylococcus aureus, Coagulase negative staphylococcus (*CNS), *Streptococcus species, E.coli, P.aureuginosa, P.heamolytica, Bacillus species, Microccus species.* These isolates were contagious environmental pathogens so that proper husbandry practice should be implemented to control and prevent contagious and environmental mastitis. This can be reduced by careful milking system (single towel) usage for each cow, disinfecting hands before milking and milking infected cows lastly, regular mastitis check up for both clinical and sub clinical mastitis, use of proper cow therapy(dry cow therapy) and culling of chronically infected animals from the herd. Gentamycine, Chloroamphenicol, Kanamaycine and Vancomycine could be the drug of choice in the present study. To avoid indiscriminate use of antibiotics in the dairy farm, antibiotic susceptibility test is needed to be performed.

Acknowledgement

The authors would like to thank Asella Regional Veterinary Laboratory staff and the farm workers for their technical assistance during the laboratory and field works.

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