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Full Length Research Paper

Study on prevalence of bovine mastitis and its major causative agents in West Harerghe zone, Doba district, Ethiopia

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A cross-sectional study was carried out in Doba district, West Harerghe, Eastern Oromiya State, Ethiopia from November 2010 to April 2011 with the aim of determining prevalence of mastitis in Zebu cows and to identify associated risk factors and major etiological agents. A total of 384 lactating Zebu cows were included in this study and out of these, 89 (23.18%) were found to be reactive by California mastitis test (CMT). Bacteriological analysis of milk samples collected simultaneously yielded positive result in 76 (19.79%) of the samples. Out of 1536 quarters examined 34 (2.21%) were blind and 152 (10.12%) were positive by CMT. Bacteriological analysis carried out at the quarter level showed that 121 (8.03%) of the samples were positive for various bacteria. The predominant bacteria isolated were *Staphylococcus* species (47.11%), *Streptococcus* species (31.40%) and coliforms (9.92%). Other bacterial species were isolated at lower rates. Antimicrobial susceptibility test showed that most of the isolates in the study area were found to be highly sensitive to cloxacillin, gentamycin and amoxicillin, and moderately sensitive to ampicillin and oxytetracycline. Nevertheless, *Streptococcus* and *Staphylococcus* species isolated were resistant to streptomycin and penicillin. Age, parity, stage of lactation and hygienic conditions were found to be important risk factors associated with the occurrence of mastitis.

Key words: Bacterial pathogens, cows, Doba, masttitis, prevalence, risk, Zebu.

INTRODUCTION

Diary production is a biologically efficient system that converts feed and roughages to milk (Yohannes, 2003). Milk has high nutritive value rich in carbohydrates, proteins, fats, vitamins and minerals. The total annual milk production of Ethiopia is estimated at 797,900 to 1,197,500 metric tons of raw milk, out of which 85 to 89% is derived from cattle. FAO (2003a) estimated that 42% of total cattle herds of Ethiopia for private holding are milking cows. Despite large number of dairy cows, milk production does not satisfy the nations' demand for milk. The per capita milk availability was estimated at 17 to 18kg per year compared to the global average of 100 kg per year (FAO, 2003b). Different constraints have been listed as causes of low milk production in Ethiopia. Among these, mastitis is the major one.

Mastitis (inflammation of mammary gland) is among important health problems in dairy cattle. It has been considered one of the most important threats affecting the dairy industry throughout the country. Mastitis is a management related disease whose prevention and control depends among other factors on good management practices (Mungube et al., 2004). It is mostly a result of combined interplay among exposure to microbes, cow defense mechanisms and environmental risk factors (Suriyasathaporn et al., 2000). The causes of

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mastitis are almost entirely infectious and bacteria though, rarely other pathogens and chemical or physical agents can be involved (Wellenberg et al., 2002). They are broadly classified into contagious and environmental agents (Quinn et al., 2002).

Economic losses due to mastitis are recognized world-wide as a major problem on dairy farms. Financial loss involved as a result of permanent loss of production in individual cows, discarded milk following antibiotic therapy, early culling of cows, veterinary costs, drug costs, increased labor, death of per acute cases and replacement costs. Bishi (1998) also reported that the economic losses from clinical and subclinical mastitis in Addis Ababa milk shed to be approximately 270 Ethiopian birr (ETB) per lactation. Mungube (2001) estimated the economic losses from mastitis in the peri-urban areas of Addis Ababa; losses due to mastitis (milk production loss, treatment cost, withdrawal losses and culling losses) were estimated to 210.8 ETB/cow/lactation from which milk production loss contributed to 38.4%. In other study conducted in the same study area by Mungube et al. (2005) was reported that a quarter with mastitis loss an average of 17.2% of its milk production.

Some studies have been conducted so far on the prevalence and major causes of bovine mastitis mostly in cross bred dairy cattle in different parts of the country (Workineh et al., 2002; Biffa et al., 2005; Sori et al., 2005). The majority of the smallholder farms in the country raise indigenous Zebu cattle. They derive milk and milk products from cattle for both home consumption and generation of income. The occurrence of mastitis in Zebu cows can affect household food security and reduce the income expected from the sector. This can affect the wellbeing of rural women in particular who use dairy products to generate their income. Information on mastitis in Zebu cows is however scanty. Particularly, no study has been carried out on mastitis in Doba districts of West Harerghe zone of Oromiya regional State, Eastern Ethiopia. In the current, attempt was made to quantify the occurrence of mastitis at cow and quarter level. Therefore, the objectives of the present study were: to determine the prevalence of bovine mastitis in Zebu cows, to isolate and identify the major etiological agents of bovine mastitis, to determine associated risk factors in the study area.

MATERIALS AND METHODS

Study area

The study was conducted in selected peasant associations (PAs) of Doba district in West Harerghe zone of Oromia regional State, Ethiopia. Doba district is located 382 km east of Addis Ababa. The daily temperature of the area ranges from 22 to 27°C. The mean annual rainfall of the area ranges from 760 and 900 mm. The average altitude of the area ranges from 1400 to 2500 m above sea level. There are about 80,796 head of cattle, 84,507 goats, 23,723 sheep, 10,899 equines, 1,894 camels and 75,305 poultry in the districts. All of these livestock species are reared by smallholder farmers under extensive production system. The district is divided into 40 administrative PAs (Doba district Agricultural and Rural development office, personal communication).

Study population and sampling method

The study animals were the indigenous lactating Zebu cows managed under extensive farming system. A simple random sampling technique was employed to select PAs, households and the animals. From the 40 PAs in the district, 8 PAs were randomly selected and milk sample was collected from lactating Zebu cows owned by selected households.

Sample size determination

For estimation of the prevalence of mastitis, since there was no work done in the study area, the sample size was determined by assuming the expected prevalence to be 50% with the 95% confidence level and desired precision of 5% using the formula described by Thrusfield (2005). Accordingly, the minimum sample size required was 384 lactating cows.

Study design and methodology

The study design used was cross-sectional study and it was carried out from November, 2010 to April, 2011. The methods employed in this study include physical examination of the udder and teats, California mastitis test (CMT), bacteriological culture and antimicrobial sensitivity tests.

Physical examination of udder

The udder was first examined visually and then thoroughly palpated to detect possible fibrosis, signs of inflammation, visible injury and tick infestation, atrophy of the tissue and swelling of the supra-mammary lymph nodes. Rectal temperature of those cows with clinical mastitis was taken to check systemic involvement. Information related to the previous health history of the mammary quarters and cause of blindness was obtained from the owners of the cows. Viscosity and appearance of milk secreted from each mammary quarter were examined for the presence of clots, flakes, blood and watery secretions.

Preparation of udder and teats

The udder, especially the teats were cleaned and dried before milk sample collection. Dust, particles of bedding and other filth were removed by brushing the surface of the teats and udder with a dry towel. The teats were swabbed with cotton soaked in 70% alcohol (Quinn et al., 1994). To prevent recontamination of teats during scrubbing with alcohol, teats on the far sides of the udder were scrubbed with alcohol first, then those on the near side.

Milk sample collection

When clinical mastitis was detected, milk samples were collected by a standard milk sampling technique as described by Quinn et al. (2002). To reduce contamination of the teat ends during sample collection, the near teats were sampled first, followed by the far ones. Approximately 10 ml of milk samples were collected into sterile test tube after discarding the first three milking streams.

Table 1. Results showing the prevalence of mastitis in Zebu cows in Doba district, West Harerghe.

No. of cows	Proportion	
	Number	%
With blind teats	34	2.22
Positive for CMT	89	23.18
Positive for bacterial growth	76	19.79
Total number examined	384	

Table 2. Results of occurrence of mastitis in Zebu cattle at the quarter level in Doba district.

Quarter	CMT positive		Culture positive	
	Number	%	Number	%
RF	39	10.16	25	6.51
RH	44	11.46	40	10.42
LF	25	6.51	16	4.20
LH	44	11.46	40	10.42
Total quarter positive	152	10.12	121	8.05

RF, Right front; RH, right hind; LF, left front; LH, left hind.

California mastitis test

Milk samples were also collected from cows in which clinical mastitis was not detected to look for sub-clinical mastitis. Milk 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. Reactions were graded as 0 and trace for negative, +1, +2 and +3 for positive (Quinn et al., 1999). 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.

Bacteriological isolation and characterization

Milk samples were culturally examined according to the procedures described by Quinn et al. (1999). In refrigerated milk samples, bacteria may be concentrated in cream layer and held within clumps of fat globules (Quinn et al., 1994). Hence, dispersion of fat and bacteria was accomplished by warming the samples at 25°C for 15 min and shook before plating on standard bacteriological media. A loop full of milk sample collected from each infected quarters was inoculated into MacConkey agar and blood agar base enriched with 7% ovine blood. The inoculated plates were then incubated aerobically at 37°C for 24 to 48 h. Identification of bacteria on primary culture was made on the basis of colony, hemolytic characteristics, gram's reaction including shape and arrangements of the bacteria, catalase and O-F test. *Staphylococci* were identified based on catalase test and growth characteristics on mannitol salt agar. Gram-negative isolates grown on MacConkey were identified based on growth characteristics on MacConkey, oxidase reaction, catalase test, triple sugar iron (TSI) agar, and the 'IMVC' test (Quinn et al., 1999).

Antimicrobial sensitivity test

In vitro antibiotic sensitivity test (Kirby-Baur disc diffusion) method was carried out in order to identify the most effective drugs for mastitis treatment in the study area. A loop full of colony from the growth of isolates was transferred to the nutrient broth in tubes and incubated at 37°C for 5 h. Mueller-Hinton agar which was used as plating medium was inoculated with broth (bacterial suspension) by using cotton swab. Then antibiotic impregnated paper disc (Oxoid, UK) were applied and pressed onto the plate with forceps. Plates were incubated at 37°C for 18 h. The diameters of zones of growth inhibition were measured in millimeter and interpreted as sensitive, intermediate and resistant to different antibiotics (Quinn et al., 1999). The drugs used were oxytetracycline, streptomycin, ampicillin, penicillin, cloxacillin, amoxacillin and gentamycin.

The obtained data was entered to Microsoft office excel 2007 program and analyzed by STATA version 11.0. The association of risk factors with occurrence of the disease was determined by Chi square (χ^2) test.

RESULTS

Physical examination of udder

Out of 384 cows examined physically for udder condition/ health, only 28 cows (7.29%) or 28 cows out of 89 mastitis positive cows for CMT (31.46%) were showed sign of fibrosis, cardinal signs of inflammation, visible injury and tick infestation, atrophy of the tissue of udder and swelling of the supramammary lymph nodes.

Prevalence of mastitis

A total of 1536 quarters from 384 milking Zebu cows were investigated. Among these, 1502 (97.78%) quarters were found to be patent while the remaining 34 (2.22%) were blind. 26 (76.47%) of these animals had single blind teat while 4 (11.76%) of them had double blind teats. Apart from the presence of blind teats, other indications of mastitis were also observed during this cross-sectional study. The results of CMT carried out on milk samples collected from those patent teats showed that 89 (23.18%) of the cows gave positive reaction. The bacteriological culture result showed that samples from 76 (19.79%) of the cows yielded bacterial agents (Table 1).

At the quarter level, 152 (10.12%) of them were positive for CMT and 121 (8.05%) of them were positive for bacterial growth (Table 2). The proportion of right front (RF), right hind (RH), left front (LF) and left hind (LH) quarters that reacted positively with CMT was 10.16, 11.46, 6.51 and 11.46%, respectively, whereas, the proportion of these quarters from which bacteria were isolated was 6.51, 10.42, 4.20 and 10.42%, respectively. The number of affected hind quarters were higher (80; 10.42%) than that of the front quarters (41; 5.47%) for bacterial growth (Table 3).

Risk factors

In this study, the risk factors of mastitis identified in Zebu cows under traditional smallholder farming system were

Table 3. Prevalence of clinical and subclinical mastitis at cow and quarter level on the basis of CMT and culture result.

Mastitis type	CMT No. (%)		Culture No. (%)	
	No. of animals	No. of quarters	No. of animals	No. of quarters
Clinical	28(7.29)	69(4.60)	28(7.29)	59(3.90)
Subclinical	61(15.89)	83(5.52)	48(12.50)	62(4.13)
Total	89(23.18)	152(10.12)	76(19.79)	121(8.03)

Table 4. Results of analysis of risk factors of mastitis in zebu cows in Doba district, West Harerghe.

Risk factor	No. examined	No. positive	χ^2	p-value
Age group (Years)				
3 - 7	161	20		
8 - 12	203	47	14.95	0.0006
Older than12	20	9		
Parity number				
1 - 3	209	27		
4 - 6	159	40	22.50	0.0001
Greater than 6	16	9		
Hygienic condition				
Poor	112	56		
Intermediate	107	12	92.55	0.0001
Good	105	8		
Lactation stage (Months)				
1 - 3	99	44		
4 - 6	86	26	79.13	0.0001
7 - 9	118	3		
Greater than 9	81	3		

parity, age, stage of lactation and hygienic conditions. Table 4 shows the results of measurement of association between mastitis and the risk factors. The result showed that the prevalence of mastitis was significantly higher (45.00%) in animals older than 12 years followed by animals in the age range of 8 to 12 years and lowest in animals younger than 8 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 (χ^2 = 22.50, P = 0.0001). It was followed by cows with parity of 4 to 6 and those with parity of 1 to 3 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 intermediate and relatively good hygienic conditions (χ^2 = 92.55, P = 0.0001). 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 1 to 3 months of lactation and it was found to decrease as lactation stage increased.

Bacteriological culture

The results of bacteriological culture on milk samples collected from mastitis Zebu cattle showed the involvement of 121 bacteria belonging to 9 genera and 13 species. Both contagious and environmental bacteria were isolated from milk samples collected from mastitis cows. The predominant bacterial species isolated was *Staphylococcus* species (47.11%), followed by *Streptococcus* species (31.40%) and *Escherichia coli* (9.92%). From both the clinical and subclinical mastitis cases, *Staphylococcus aureus* and *Streptococcus agalactie* were the predominant bacteria isolated during this study (Table 5).

Table 5. Results of bacteriological culture of milk samples from Zebu cows with mastitis in Doba district.

Organism	Number of isolates (%)		
	Clinical mastitis	Subclinical mastitis	Total
S. aureus	25 (20.70)	18 (14.90)	43 (35.5)
S. agalactie	12 (9.9)	12 (9.9)	24 (19.9)
Staphylococcus intermidius	6 (4.9)	2 (1.6)	8 (6.6)
Escherichia coli	3 (2.5)	4 (3.3)	7 (5.8)
Streptococcus disaggalactie	4 (3.3)	3 (2.5)	7 (5.8)
Streptococcus uberis	4 (3.3)	3 (2.5)	7 (5.8)
Staphylococcus hycus	1 (0.8)	5 (4.1)	6 (4.9)
Pseudomonas aeroginosa	-	6(4.9)	6 (4.9)
Corynebacterium bovis	-	5 (4.1)	5 (4.1)
Klebsiella ssp	2 (1.6)	1 (0.8)	3 (2.5)
Pasteurella haemolytica	1 (0.8)	1 (0.8)	2 (1.6)
Proteus spp	1 (0.8)	1 (0.8)	2 (2.7)
Bacillus cereus	-	1(0.8)	1 (0.8)
Total	59	62	121

Table 6. Results of antimicrobial sensitivity tests on the bacterial isolates.

Pathogen	Total isolate	No. tested	OXY	GEN	STR	AMP	AMO	PEN	CLO
S. aureus	43	25	13/12	22/3	8/17	13/12	21/4	6/19	24/1
S. agalactie	24	16	9/7	14/2	6/10	8/8	15/1	4/12	14/2
S. intermidius	8	5	2/3	4/1	3/2	3/2	4/1	3/2	4/1
S. disagalacti	7	7	3/4	5/2	3/4	3/4	5/2	1/6	6/1
S. uberis	7	5	2/3	5/0	2/3	3/2	4/1	1/4	4/1
E. coli	7	4	2/2	3/1	3/1	2/2	3/1	1/3	3/1
Total	96	62	31/31	53/9	25/37	32/30	52/10	16/46	55/7

The numerators show the number of sensitive isolates, while the denominator show the number of resistant isolates among tested. OXY, Oxytetracycline (30 µg); GEN, gentamicin (5 µg); AMO, amoxicillin (10 µg); STR, streptomycin (10 µg); AMP, ampcillin (2 µg); PEN, penicillin (10 IU); CLO, cloxacillin (5 µg); S/R, sensitive/resistance.

Antimicrobial sensitivity test

A total of 62 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 cloxacillin, gentamicin and amoxicillin (Table 6). *E. coli* were moderately sensitive to oxytetracycline and ampicillin whereas *Staphylococcus* and *Streptococcus* species were highly resistant to streptomycin and penicillin.

DISCUSSION

Zebu cows are of a great economic importance for rural smallholder dairy enterprise throughout Ethiopia. However, loss or reduction of milk production as a result of mastitis has been known in most dairy farms raising cross bred or exotic cattle. The epidemiology of mastitis has never been investigated in Zebu cattle. This study attempted to investigate into the prevalence of mastitis in Zebu cattle in the rural area of West Harerghe. The prevalence recorded in this study (23.18%) is significant at the cow level. This finding is lower than those of Mekbib et al. (2009), who reported 71% in exotic dairy cows of Holeta farm. Tola (1996) reported a rate of 61.11% and Zarihun (1996), reported 68.10% elsewhere in Ethiopia in cross bred dairy cattle. Our finding on prevalence of mastitis are also lower than that of Rahman et al. (2010), who reported 53.30% prevalence in dairy cows in Bangladesh. 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. The previous studies earlier mentioned were carried out on exotic and cross bred cows, while our study includes only Zebu cows. Zebu cattle have been known for their relative resistance/ tolerance against many infectious diseases. Our study focuses on Zebu cows owned by smallholder farmers who

own few cows. This might have decreased the potential of contagious mastitis causing pathogens and made easier to clean the cows' environment and in turn might have also decreased mastitis causing environmental pathogens. As mastitis is a complex disease, involving interactions of various factors which include management, environmental, animal risk factors and causative agents, its prevalence will vary (Radostits et al., 2007).

The overall quarter level prevalence recorded in this study (8.03%) is lower than that of Mekbib et al. (2009), Nesru et al. (1999) and Biffa et al. (2005) who reported 44.80, 37 and 28.20%, respectively. From the 152 CMT positive quarter milk samples, 121 (79.60%) were bacteriologically positive up on culturing, while 31 (20.40%) were bacteriologically negative, which is in line with the results of Aregaw (1992), who reported 18% bacteriologcally negative samples. Our finding is higher than that of Sori et al. (2005) and Biffa (1994). The failure of isolation of bacterial agents from CMT positive quarter milk be due the predominance of subclinical mastitis in the area. Since the farms studied were mainly found in the rural area where veterinary services are not adequate, most farmers use treatment on their own or get the services from paraveterinarians. Lack of stringency in provision of therapy can possibly change the clinical cases into subclinical mastitis. The administration of antibiotics can suppress the bacterial agents and inhibit their growth in the media. It could also be due to spontaneous elimination of infection, intermittent shedding of pathogens, intracellular location of pathogen and the presence of inhibitory substances in milk (Radostits et al., 2007). It might also be due to some cases of delayed healing of infection from which organism may disappear or reduced, while infiltration of leukocytes continued until healing is completed (Sori et al., 2005).

The results of analysis of the supposed risk factors showed that age was one of the predisposing factors. The occurrence of more cases of mastitis in older animals observed in the present study is in agreement with reports of Biffa et al. (2005). Biffa et al. (2005) have also found strong association between age and pre-valence of mastitis. 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 (Harmon, 1994; Radostits, 2003). 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 in consent with findings of Sergant et al. (1998), Bustan et al. (2000) and Kerro and Tareke (2003). 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 defense mechanisms. Repeated parturition also exposes cows to environmental and contagious bacteria. Besides, multiple parturition stresses cows and ultimately down regulates their immunity. In general, the immunity animals decrease through age making older animals more prone to mastitis.

The prevalence of mastitis was higher in cows in early lactation as compared to those in late lactation. In support of our results previously, Nesru et al. (1999) and Kerro and Tareke (2003) reported higher prevalence of mastitis during early lactation than late lactation from different parts of Ethiopia. The results of Thennarasu and Muralidharium (2004) carried out elsewhere also supports our findings. The occurrence of more cases during earlier lactation stage may be due to absence of dry cow therapy and birth related influences (Quinn et al., 2005). The amount of milk ejected is also higher during earlier lactation periods and this cause increased in patency of the teats and decreased local defense factors. Similarly, Kerro and Tareke (2003) and Radostitis (2003) suggested that the mammary gland is more susceptible to new infection during early lactation and late dry period, which may be due to the absence of udder washing and teat dipping, which may in turn increase the number of potential pathogens on the skin of the teat. In line with this, Quinn et al. (2005) asserted that many infections caused by environmental pathogens occur during the dry off period and on the week before calving. The prevalence of mastitis was higher in animals kept on farms with poor hygienic conditions and in those who practice poor milking hygiene. This well agrees with Mekbib et al. (2009) who reported higher prevalence of mastitis in animals kept on farms with poor hygiene. This may be due to increased exposure and transmission of pathogens during milking (Kivaria et al., 2004).

The result obtained from bacteriological analysis of the sample revealed that the predominant organism isolated from mastitis milk were *S. aureus* followed by *S. agalactia*. This is comparable with the observations of Zerihun (1996), Geresu (1989) and Nesru et al. (1999) from Ethiopia and that of Madut et al. (2009) in cows from Sudan. The predominance of these two bacterial species is due to frequent colonization of teats as they are commensals of the skin. Then they can easily get access to the teat canal during milking or suckling and can be transmitted from quarter to quarter and from cows to cows during milking practices. Their ability to exist intracelullarly and localize within micro-abscessation in the udder and hence, resistant to antibiotic treatment (MacDonald, 1997) could also be important factor contributing to the predominance of these organisms. However, the incidence of the *S. agalactia* in this study is greater than that of Mekbib et al. (2009) and lowers than that reported by Tolassa (1987) and Mekuria (1986) who found *Streptococcu*s species to be 53.55 and 45.50%, respectively.

The third predominant bacterial species isolated were the coliforms. This finding is comparable with the results

of Kerro and Tareke (2003), in which the coliform accounts for 14.10% and were the third predominant pathogens from dairy cows in Southern Ethiopia. *E. coli* were the predominant bacteria among the coliforms with an isolation rate of 5.60% in this study which is in consent with the observations of Mekbib et al. (2009), Mekuria (1986) and Biffa (1994) who reported 4.60, 3.64 and 3.14%, respectively from different parts of Ethiopia. In this study, *Klebsiella* spp accounted for 2.50% among coliforms which is in agreement with Mekbib et al. (2009) who reported 3.30%.

The *in vitro* disc sensitivity test showed that cloxacillin is the most effective drug followed by gentamycin and amoxicillin in the study area. Similar results have been published by Sumathi et al. (2008) in support of our findings who reported gentamycin to be the drug of choice in Bangalore. However, *Staphylococcus* and *Streptococcus* species showed the existence of resistance to streptomycin and penicillin. These two antibiotics are most widely used in many parts of Ethiopia. They are the most commonly available and affordable antibiotics to farmers under Ethiopia condition. They are sometimes the only available antibiotics in many veterinary clinics. It might be this wide use of these drugs and inappropriate administration which have contributed to the development of resistance by the predominant bacterial agents in the area. The prevailing subclinical mastitis in the area also supports this hypothesis. The effectiveness of cloxacillin detected in this study agrees with findings of Quinn et al. (2005) who asserted that cloxacillin is an effective drug against *S. aureus*, *S. agalacteae*, *E. coli* and environmental streptococci. Bacteria may manifest resistance to antibacterial drugs through a variety of mechanisms. Some species of bacteria are innately resistant to ≥1 class of antimicrobial agents. In such cases, all strains of that bacterial species are likewise resistant to all the members of those antibacterial classes. Of greater concern are cases of acquired resistance, where initially susceptible populations of bacteria become resistant to an antibacterial agent and proliferate and spread under the selective pressure of use of that agent. Several mechanisms of antimicrobial resistance are readily spread to a variety of bacterial genera. Through genetic exchange mechanisms, many bacteria have become resistant to multiple classes of antibacterial agents, and these bacteria with multidrug resistance (defined as resistance to ≥3 antibacterial drug classes) have become a cause for serious concern, particularly in hospitals and other healthcare institutions where they tend to occur most commonly (Fred, 2006).

In conclusion, mastitis was found to be among standing problems of cattle production in the area. Age, parity number, stage of lactation and hygienic conditions were important risk factor associated with mastitis in Zebu cows in Doba district. *S. aureus, S. agalactiae* and *E. coli* were the most important bacterial agents of mastitis.
Resistance was detected in the predominant bacterial agents against most commonly used antibiotics.

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