

Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia

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Abstract A study was conducted to determine the prevalence of mastitis, identify the major bacterial pathogens and test the antimicrobial resistance of milk bacterial isolates in smallholder dairy farms in Selalle area, Ethiopia. A total of 109 smallholder dairy farms comprising 500 crossbred lactating cows were included. The prevalence of clinical mastitis at herd, cow and quarter level was 8.3% (n=9), 1.8% (n=9) and 0.51% (n=10), respectively, while that of sub-clinical mastitis was 54.7%, 22.3% and 10.1%, respectively. The univariate logistic regression showed that among the risk factors considered, presence of teat lesion, stage of lactation and parity number had significant effect on the prevalence of sub-clinical mastitis. However, after multivariate analysis, only presence of teat lesion and stage of lactation had significant effect. The common isolates from the clinical mastitic quarters were *St. agalactiae* (30%, n=3) and *St. dysgalactiae* (30%, n=3), while from sub-clinical cases were *S. aureus* (42.6%, n=83), *S. epidermidis* (22.1%, n=43), *St. agalactiae* (12.8%, n=25) and *St.*

uberis (10.3%, n=20). *Staphylococcus intermedius* and *Streptococcus dysgalactiae* were the species, which showed high level of susceptibility for most of the antimicrobials tested, while the remaining had varying levels of resistance for almost all the antimicrobials used. Among the antimicrobials employed, erythromycin and sulphonamide showed the lowest proportion of resistant isolates. Considering the possible significant economic losses that could be incurred by both clinical and sub-clinical mastitis, attention should be paid for further detailed investigations including the economic losses and benefits of interventions in the study area.

Keywords Antibiotic resistance · Bovine · Mastitis · Prevalence · Smallholder

Introduction

Ethiopia holds large potential for dairy development due to its large cattle population and the favorable climate for improved high yielding animal breeds. Thus, the contributions of the dairy sector especially the smallholder system in Ethiopia to poverty alleviation and sustainable food production in the country is assumed to be considerable, given the considerable potential for income and employment generation from high value dairy products. However, among many factors the sector is constrained by mastitis, which incurs serious economic losses to the dairy industry.

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A number of previous reports from different parts of the country indicated that mastitis is a serious problem in the dairy industry of Ethiopia (Biru, 1989; Bishi, 1998; Nesru, 1999; Mungube, 2001). However, little has been done in Selalle area, which is located within the Addis Ababa milk-shed, supplying a considerable volume of milk to government and private milk processing plants and having a high potential for dairy production. Thus, this study was initiated to estimate the prevalence of mastitis, isolate the major bacterial pathogens and test their antimicrobial susceptibility and assess the major risk factors associated with the occurrence of mastitis in smallholder dairy farms in the Selalle area.

Materials and methods

Study area and study population

The study was conducted in Mullo-Sullulta District of the North Shoa (Sellale area) Zone of Oromia, which is located some 40 km northwest of the capital, Addis Ababa. The total area coverage of the Selalle area is 11607 km², while the altitude ranges from 1000 – 3500 meter above sea level (CSA, 2003). The mean annual rainfall of the area is 1026 mm and the daily mean maximum and minimum temperatures are 20.7 and 11.2 °C, respectively. The total cattle population of the Zone is estimated to be 1,173,543; while that of Mullo-Sullulta District is 186,136 out of which 6,593 (3.5%) heads of cattle are crosses of Holstein and indigenous breeds. The smallholder dairy farms in the Mullo-Sullulta District represent the study population.

Study design and sampling procedure

A cross-sectional type of study was carried out to investigate the prevalence of clinical and sub-clinical mastitis at quarter, cow and herd level. Simple random type of sampling was carried out to select peasant associations and farms. All dairy cows in the selected farms were sampled as a cluster. The total number of animals sampled from the study area is calculated using the formula for one stage cluster sampling (Thrusfield, 1995). The considerations during the sample size determination included: 95% confidence interval, 5% precision and 60% of prevalence from previous studies in the same study

area (Workineh et al., 2002). Accordingly, a total of 109 dairy farms comprising 500 lactating crossbred cows were included in the study.

Clinical examination and California Mastitis Test (CMT)

The selected smallholder dairy farms were visited once (when the questionnaire survey, clinical examination and milk sampling were done in one visit) or twice in few cases. Crossbred milking cows were clinically examined for the diagnosis of mastitis. Clinical mastitis was diagnosed on the basis of manifestation of visible signs of inflammation. A quarter, which was warm and swollen and had pain upon palpation was considered to have acute clinical mastitis, while misshaped, atrophied, hard and fibrotic quarter was considered to have chronic mastitis (IDF, 1987).

The California Mastitis Test (CMT) was carried out following the procedure described by Quinn et al. (1994) as screening test for sub-clinical mastitis and for further bacteriological examination. The test result was interpreted based on the thickness of the gel formed by CMT reagent and milk mixture and scored as negative (o), trace (T), + (weak positive), ++ (distinctive positive), and +++ (strong positive). Quarters with CMT score of + or above were judged as positive. Cows were considered positive for CMT, when at least one quarter turned out to be positive for CMT. A herd was considered positive for CMT, when at least one cow in a herd was tested positive with CMT.

Collection of milk samples

Milk samples were collected from all the quarters (unless they were blind) of the sampled cows by standard milk sampling techniques (Sears et al., 1993; Quinn et al., 1994). After a quarter had been washed with tap water and dried, the teat end was swabbed with cotton soaked in 70% ethyl alcohol. Approximately 10 ml of milk was collected into a sterile universal vial after discarding the first three milking streams. Samples were placed in an icebox and transported to the National Animal Health Research Center at Sebeta and stored at +4°C for a maximum of 24 hours (h) until they were inoculated into standard bacteriological media.

Bacteriological examination of milk samples

Milk samples were examined following the standard procedures (Sears et al., 1993; Quinn et al., 1994). About 10 µl of each milk sample was streaked on 5% blood agar. The inoculated plate then was incubated aerobically at 37 °C for up to 72 h. The plates were examined for growth, morphology and hemolytic features at 24, 48 and 72 h after inoculation. Plates were considered culture negative, if no growth occurred within 72 h. Bacteria on culture-positive plates were identified according to their gram-stain reaction, colony morphology and hemolytic features. Based on the above results, suspected colonies were isolated on to blood agar plates for further investigation. Then growth on MacConkey agar, catalase test, oxidase test, O-F test and motility test were used for primary identification and Indole test, Methylene Red test, Voges-Proskauer test and Urease test were used as secondary biochemical tests (Sears et al., 1993; Quinn et al., 1994). The interpretation was made as provided by National Mastitis Council (NMC, 1990). Isolation of two (if neither of the two colonies is a recognized pathogen) or more types of colonies from a quarter sample were considered as contaminated and the results were disregarded.

Antimicrobial sensitivity test

Kirby-Bauer disk diffusion method (Carter and Chengappa, 1991) was employed to test the in-vitro antibiotic sensitivity of the isolates. The following eight antimicrobial drugs were used: ampicillin (AMP) (10 µg), penicillin G (PEN) (10IU), streptomycin (STR) (10 µg), kanamycin (KAN) (30 µg), erythromycin (ERY) (15 µg), polymixin B (PB) (300 µg), oxytetracycline (OXTE) (30 µg), and sulfonamide (SULPHA) (300 µg). The cut off values for the evaluation of the susceptibility of isolates were when the inhibition zone was greater than or equal to 29 mm, 30 mm and 17 mm for ampicillin (for staphylococci, streptococci and other microorganisms, respectively), 29 mm and 22 mm for penicillin G (for staphylococci and other microorganisms, respectively), 15 mm for streptomycin, 18 mm for kanamycin and erythromycin, 12 mm for polymixin B, 19 mm for oxytetracycline and 17 mm for sulphonamide. As plating medium Mueller-Hinton agar and 5% sheep blood agar were used for less fastidious bacterial

isolates and streptococci species, respectively. Antibiotic impregnated paper discs and plates were incubated at 37°C for 16–18 h.

Questionnaire survey

A structured questionnaire (pre-tested) with the primary objective of elucidating the multi-factorial background of bovine mastitis was conducted. Information regarding cow and farm attributes, which included parity number, stage of lactation (classified into three: beginning of lactation referring to the first two months of lactation period; middle of lactation referring to the next five months period and end of lactation referring to the last weeks of lactation period), herd size, barn floor status (good if the farm floor is made of concrete and bad if the floor is muddy), milking hygiene (good if it fulfilled either of the two of the practices including washing of hand before milking, use of separate towel for each lactating cow and drying of udder after washing and bad if not) and sequencing milking of mastitic cows were collected.

Statistical analysis

Prevalence rates were calculated for clinical and sub-clinical mastitis at herd, cow, and quarter level as defined by the CMT score and bacteriological result. The prevalence of sub-clinical mastitis was the dependent variable while parity, stage of lactation, herd size and presence of teat lesion were independent variables considered at cow level. The independent variables at herd level included barn floor status, milking hygiene and milking strategy. The associations between dependent and independent variables were tested by logistic regression of Intercooled Stata 7.0 (STATA, 2000).

Results

Prevalence of clinical and sub-clinical mastitis

Among the total of 2000 quarters of the 500 lactating cows, 45 quarters (2.3%) belonging to 37 cows were blind and 17 quarters (0.9%) belonging to 16 cows were affected by clinical mastitis. From cows having blind quarters, 30 (81.1%) cows had only one blind

quarter, 6 (16.2%) cows had two blind quarters and 1 (2.7%) cow had three blind quarters. Fifteen (33.3%), 11 (24.6%), 10 (22.2%), and 9 (20.0%) of the blind quarters were at the right rear, right front, left rear and left front positions, respectively.

A total of 14 (12.8%) herds, 16 (3.2%) cows and 17 (0.9%) quarters had clinical mastitis while 77 (70.6%) herds, 147 (30.4%) cows and 264 (13.6%) quarters had sub-clinical mastitis. The details of prevalence rates of clinical and sub-clinical mastitis and their respective bacterial culture results at herd, cow and quarter levels are presented in Table 1. Out of the 17 quarters, which were affected by clinical mastitis, 4 (23.5%) and 13 (76.4%) were acute and chronic form of mastitis, respectively. Mastitis causing bacteria were isolated in 9 herds (8.3%), 9 cows (1.8%) and 10 quarters (0.5%) in clinical cases, while the isolation rate was 59 (54.7%) at herd level, 108 (22.3%) at cow level and 195 (10.1%) at quarter level from sub-clinical cases.

From the CMT negative quarters ($n=1674$), 70 (4.2%) were with trace reaction while from the CMT positive quarters (13.6%, $n=264$), 4.1% ($n=80$), 4.1% ($n=79$), and 5.4% ($n=105$) were weak, moderate and strong reactors, respectively. Seventy-one (26.9%), 67 (25.4%), 67 (25.4%) and 59 (22.3%) of the CMT positive quarters were found in the right rear, right front, left rear and left front quarters, respectively. The difference in prevalence between the quarters was not statistically significant ($p>0.05$).

Risk factors associated with sub-clinical mastitis

The univariate logistic regression analysis revealed that stage of lactation ($p<0.05$), parity number ($p<0.05$) and presence of teat lesion ($p<0.01$) had significant effect on the prevalence of sub-clinical mastitis at cow level. These risk factors that had

significant effect in univariate analysis were fitted in a multivariate model and only stage of lactation ($p<0.05$) and presence of teat lesion ($p<0.01$) had significant effect. The prevalence rates were higher in cows that were at the end of their lactation period ($OR=1.5$) and with teat lesions ($OR=6.2$) (Table 2). All the farm attributes considered (barn floor status, milking hygiene and milking of mastitic cows at last) had no significant effect on the prevalence of sub-clinical mastitis at cow level. Moreover, the effects of the potential risk factors at herd level were analyzed using univariate logistic regression and the results revealed that none of the factors considered had significant effect on the prevalence of sub-clinical mastitis at herd level ($p>0.05$) (Table 2).

Bacterial isolates

The list, number and proportion of the bacterial isolates from a total of 117 cows (205 quarters) are presented in Table 3. A total of 205 isolates were found, of which 10 (4.9%) were from milk samples collected from clinical mastitic quarters, while the remaining 195 (95.1%) were from sub-clinical cases. From sub-clinical cases, 191 (97.9%) isolates showed single growth, while 4 (2.1%) had mixed growths. The common isolate from the clinical mastitic quarters were *St. agalactiae* (30%, $n=3$) and *St. dysgalactiae* (30%, $n=3$), while from sub-clinical cases were *S. aureus* (42.6%, $n=83$), *S. epidermidis* (22.1%, $n=43$), *St. agalactiae* (12.8%, $n=25$) and *St. uberis* (10.3%, $n=20$). Other isolates include *S. intermedius*, *St. dysgalactiae*, *Micrococcus species*, *B. cereus*, *A. pyogenes*, *E. coli*, and *C. bovis*.

Based on their importance, 8 (80%) and 134 (70.2%) of the isolates from the clinical and sub-clinical mastitic cases, respectively were major pathogens, while the remaining were minor pathogens.

Table 1 Prevalence of clinical and sub-clinical mastitis at herd, cow and quarter levels in Selalle smallholder crossbred lactating cows, central Ethiopia

Observation level	Clinical mastitis			Sub-clinical mastitis		
	No.	+ve cases No. (%)	+ve cultures No. (%)	No.	CMT +ve No. (%)	+ve cultures No. (%)
Herd level	109	14 (12.8)	9 (8.3)	109	77 (70.6)	59 (54.7)
Cow level	500	16 (3.2)	9 (1.8)	484	147 (30.4)	108 (22.3)
Quarter level	1955	17 (0.9)	10 (0.5)	1938	264 (13.6)	195 (10.1)

+ve=positive; No.=number; CMT=California Mastitis Test

Table 2 Association of risk factors with the prevalence of sub-clinical mastitis at cow and herd levels (logistic regression) in Selalle smallholder crossbred lactating cows, central Ethiopia

Risk factors	Groups	N	Prevalence (%)	Univariate analyses P-value	Multivariate analyses		
					P-value	OR	CI
Stage of lactation	Beginning	86	16.3	0.016	0.020	1.5	1.06–2.04
	Middle	242	20.2				
	End	156	28.8				
Parity number	1–3	334	19.8	0.045	0.079	1.5	0.95–2.37
	>3	150	28.0				
Herd size	1–3	115	26.1	0.267			
	> 3	369	21.0				
Presence of teat lesion	Yes	11	63.6	0.003	0.004	6.2	1.77–21.90
	No	473	21.4				
Barn floor status	Good	332	22.3	0.984			
	Bad	152	22.4				
Milking hygiene	Good	240	22.1	0.904			
	Bad	244	22.5				
Milking mastitic cows at last	Yes	193	22.8	0.835			
	No	291	22.0				

N= Number of observations, CI= 95% confidence interval

Among the major pathogens the predominant isolate was *S. aureus* (61%) and that of minor pathogens was *S. epidermidis* (78.2%). Based on their origin, 80% and 60.7% of the isolates from the clinical and sub-clinical mastitic cases, respectively, were contagious pathogens, while the remaining were environmental pathogens. Among the contagious pathogens, the predominant isolate was *S. aureus* from both clinical (77%) and sub-clinical (71.6%) cases. Among the environmental pathogens, *S. epidermidis* (57.3%) was the most frequently isolated bacteria from the sub-clinical cases.

In-vitro antimicrobial susceptibility test

The results of antimicrobial sensitivity test for the different isolates are presented in Table 4. Isolates of *S. aureus* showed moderate to very high resistance to ampicillin (53.4%), penicillin 45.3%, streptomycin (63.4%) and polymixin B (97.7%). *S. epidermidis* isolates were highly resistant to polymixin B (95.2%), oxytetracycline (83.3%) and ampicillin (70.8%). *St. agalactiae* isolates showed the highest resistance for kanamycin (53.8%). Substantial isolates of *St. uberis* were susceptible for most of the tested antimicrobials

Table 3 Bacterial isolates from clinical and sub-clinical mastitic milk samples in Selalle smallholder crossbred lactating cows, central Ethiopia

Type of isolates	Clinical No. (%)	Sub-clinical No. (%)	Total No. (%)
<i>S. aureus</i>	2 (20.0)	83 (42.6)	85 (41.5)
<i>S. epidermidis</i>	1 (10.0)	43 (22.1)	44 (22.5)
<i>S. intermedius</i>	1 (10.0)	3 (1.5)	4 (2.0)
<i>Micrococcus spp</i>	-	4 (2.1)	4 (2.0)
<i>St. agalactiae</i>	3 (30.0)	25 (12.8)	28 (13.7)
<i>St. dysgalctiae</i>	3 (30.0)	3 (1.5)	6 (2.9)
<i>St. uberis</i>	-	20 (10.3)	20 (9.8)
<i>B. cerus</i>	-	4 (2.1)	4 (2.0)
<i>A. pyogenes</i>	-	4 (2.1)	4 (2.0)
<i>E. coli</i>	-	1 (0.5)	1 (0.5)
<i>C. bovis</i>	-	1 (0.5)	1 (0.5)
<i>Mixed growths</i>	-	4 (2.1)	4 (2.0)
Total isolates	10 (100)	195 (100)	205 (100)

No.= number of milk samples positive for the specific bacterial isolate; %=proportion from the total of the same column.

Table 4 In-vitro antimicrobial susceptibility test results of milk bacterial isolates in Selalle smallholder crossbred lactating cows, central Ethiopia

Isolates	No.	Responses to application of antimicrobial disks (susceptible in %)							
		AMP	PEN	KAN	ERY	PB	STR	OXYTE	SULPHA
<i>S. aureus</i>	85	45.3	53.4	96.5	95.3	2.3	36	76.7	61.3
<i>S. epidermidis</i>	24	29.1	54.1	95.8	91.7	4.8	54.1	16.7	82.2
<i>S. intermedium</i>	3	100	100	100	100	100	100	100	75
<i>St. agalactiae</i>	13	84.5	84.6	46.2	92.5	92.4	84.5	70.5	92.3
<i>St. dysgalactiae</i>	3	100	100	66.6	66.6	100	100	100	100
<i>St. uberis</i>	19	63.2	65.5	31.5	100	100	92.3	92.5	69.8
<i>B. cerus</i>	4	-	-	50	50	25	75	75	75
<i>A. pyogenes</i>	3	33.3	100	-	-	66.6	-	33.3	66.6

No.=number of observations; AMP=ampicillin; PEN=pencillin G; KAN=kanamycin; ERY=erythromycin;

PB=polymixin B; STR=streptomycin; OXYTE=oxytetracycline; SULPHA=sulphonamide.

except kanamycin. Among the antimicrobial agents tested, erythromycin (except for *A. pyogenes*) and sulphonamides showed the lowest proportion of resistant isolates of all the isolated bacteria. Generally, the current antimicrobial sensitivity test indicated that the responses of the various milk bacterial isolates to different antimicrobial agents were variable.

Discussion

The overall prevalence of clinical mastitis and subsequent culture results at cow level in this study (2.6%) is in agreement with the reports of Mungube (2001) (3.6%) and Bishi (1998) (4.4%) in peri-urban and urban production systems in Ethiopia. The overall prevalence of sub-clinical mastitis (22.3%) is also in agreement with the reports of Nesru et al. (1997) (19%) in local and exotic cattle in Ethiopia and that of Klastrup and Halliwell (1997) (17–19%) in Malawi. However, it is lower than those of Abaineh and Sintayehu (2001) (34.6%) and Sori et al. (2005) (36.7%) in local and crossbred dairy cows in the central highlands and Workineh et al. (2002) (45.4%) in commercial farms in Ethiopia. Our finding is by far lower than the reports of Kerro and Tareke (2003) in local, Friesian and Jersey cows in Southern Ethiopia (62.9%) and Kivaria et al. (2004) (90.3%) in lactating cows in smallholder farms in Tanzania. Variations in herd size, management practices, proportion of exotic gene inheritance, agro-climates and other risk factors might have contributed to the observed differences in

prevalence rates of mastitis among the findings of the various workers.

The farm attributes considered in the current study (barn status, milking hygiene and milking mastitic cows at last) did not have significant effect on the prevalence of sub-clinical mastitis. However, the importance of these farm attributes in determining the prevalence of mastitis was indicated by Kivaria et al. (2004), Sori et al. (2005) and Mungube et al. (2004). The homogeneity of the production environment under smallholder's condition and the little difference in farm hygienic practices could have contributed for the lack of significant effect of the farm attributes.

The significant effect of stage of lactation on prevalence of sub-clinical mastitis in this study was also reported by Nesru (1999), Mungube et al. (2004), Kerro and Tareke (2003) and Biffa et al. (2005) in Ethiopia. The former two authors reported higher prevalence of sub-clinical mastitis for cows in mid and late stage of lactation as it is the case in our findings, while the later two reported higher prevalence in early stage of lactation. The variations in the effect of stages of lactation between the different studies could be related probably to the disparities in age, parity and breed of the sampled animals. In the current work, the prevalence of sub-clinical mastitis was significantly higher in cows with teat lesions. Similar findings were reported by Sori et al. (2005), Kerro and Tareke (2003) and Biffa et al. (2005) where the prevalence of sub-clinical mastitis was significantly higher in cows with teat lesions.

The isolation of substantial number of staphylococcal (66.2%) and streptococcal (24.6%) species as causes of sub-clinical mastitis in this study is in agreement with the findings of Workineh et al. (2002), Sori et al. (2005), Kerro and Tareke (2003) and Abdella (1996) in Ethiopia and Mdegela et al. (2005) in Tanzania. The common and most important isolate found in this study was *S. aureus* (42.6%), which is similar to the findings of Nesru et al. (1997), Abdella (1996) (47%) and Sori et al. (2005) (44%). The high prevalence *S. aureus* may be attributed to wide distribution of the organisms in teat canals and lesions, on teat and udder skin, and also in infected udder (McDonald, 1997; Jones et al., 1998). It is difficult to eliminate the bacteria from the mammary gland due to the very low rate of self cure and a number of factors affect the rate of cure after treatment which is in general low (Chamings, 1984; Sol et al., 1995).

The antimicrobial sensitivity test indicated that the responses of the various milk bacterial isolates to different antimicrobial agents were variable. Among the isolated pathogens, *S. aureus* and *S. epidermidis* were more susceptible to kanamycin and erythromycin and showed strong resistance to polymixin B. The same kinds of susceptibility pattern results were reported by Nesru (1997) in Ethiopia and Mdegela et al. (2005) in Tanzania. The variations in susceptibility of the isolated pathogens to the different drugs may be attributed to the prevailing differences on frequency and type of antibiotic treatments employed at the smallholder level. A previous report indicated that the repeated use of antimicrobials against pathogens causing sub-clinical mastitis increases selection pressure for development of drug resistant bacterial strains (Pyorala and Vesa, 1995). Furthermore, it is an established fact that *Staphylococcus* species produce β -lactamase, which can cleave the β -lactam ring of Beta-lactam antibiotics (Brumfitt and Hamilton-Miller, 1989). Interestingly, among the antimicrobial agents tested, erythromycin (except for *A. pyogenes*) and sulphonamides showed the least proportion of resistant isolates of all the bacteria tested. This finding is most probably attributed to the rare use of the drugs in the treatment of bovine mastitis in Ethiopia. On the other hand, the other antibiotics tested are employed for the treatment of mastitis more frequently due to their availability and price by smallholder farmers in Ethiopia.

Conclusion

In view of our findings and similar previous studies, the prevalence of mastitis is moderate and major pathogenic microorganisms were involved in both clinical and sub-clinical mastitis in the study area. Cow attributes including presence of teat lesion and stage of lactation influenced the prevalence of sub-clinical mastitis. It is essential thus for the smallholder dairy owners in the study area to avoid teat injury, monitor the udder health status regularly and implement control strategies as required. Awareness should also be created among smallholder farmers about the economic impacts and benefits of controlling mastitis. The antimicrobial sensitivity test showed most milk bacterial isolates including the major pathogens had multiple but variable resistance pattern. Therefore, farmers need to be advised to avoid the frequent use of one type of antimicrobial for a long period and the need to consult animal health professionals for prescription and administration of drugs.

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