A Cross-Sectional Study on the Prevalence, Antimicrobial Susceptibility Patterns, and Associated Bacterial Pathogens of Goat Mastitis

Assefa Wakwoya, MSc[1]
Bayleyegn Molla, PhD[2,3]
Kelay Belihu, PhD[2]
Josef Kleer, PhD[3]
Goetz Hildebrandt, PhD[3]

[1]*Regional Agricultural Bureau of Oromia*
Veterinary Services Division
Alem Teferi, Ethiopia

[2]*Faculty of Veterinary Medicine*
Addis Ababa University
Debre Zeit, Ethiopia

[3]*Institut für Lebensmittelhygiene*
Freie Universität Berlin
Berlin, Germany

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ABSTRACT

A cross-sectional study on mastitis in lactating goats was undertaken to estimate the prevalence, associated bacterial pathogens, and their antimicrobial resistance patterns in the Southern Rift Valley Region of Ethiopia. Of the 340 lactating goats physically examined, 8 (2.4%) showed clinical mastitis. Among the 680 milk samples collected from the 340 lactating goats, 278 (40.9%) were California mastitis test positive, and on culturing, 250 (89.9%) yielded bacterial growth. The main bacterial pathogens isolated were coagulase-negative staphylococci (CNS) (9.6%), *Staphylococcus aureus* (12.8%), and *Bacillus* (13.8%) and *Corynebacterium* (10.9%) spp. Other bacteria isolated include *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Escherichia coli*, *Pasteurella* (*Mannheimia*) *haemolytica*, and *Micrococcus* spp. Among the bacterial pathogens examined for resistance, *S aureus* (7.1%), CNS (12.4%), *Corynebacterium* (8.9%), *Streptococcus* (21.4%), *Bacillus* (37.8%) spp., *E coli* (40.4%), *K pneumoniae* (84.4%), and *E aerogenes* (83.3%) were resistant to 1 or more of the antimicrobials tested. A relatively high level of resistance was observed mainly to cloxacillin, methicillin, erythromycin, chloramphenicol, streptomycin, and oxytetracycline, suggesting the need for the prudent use of antimicrobials in animal health and productions sectors in the study areas.

INTRODUCTION

Mastitis is still one of the most economically important diseases for the dairy industry worldwide irrespective of the species of animals.[1] Mastitis can cause devastating effects to farmers because of the serious economic losses and the danger that the bacterial contamination of milk from affected goats may render it unsuitable for human consumption.[2] Contaminated milk can also serve as sources of a number of milk-borne infections to humans. In the various regions of Ethiopia, raw milk of goats is widely consumed mainly by the pastoralists.[3] Previous studies conducted in different countries indicated the distribution, causes, and importance of mastitis in dairy goats.[4-10] On the other hand there are currently very limited reports on antimicrobial resistance of mastitis pathogens isolated from dairy goats worldwide.[11-13] Studies dealing with mastitis and antimicrobial resistance in small ruminants in Ethiopia are also very rare. The present study was undertaken to provide information on the prevalence of clinical and subclinical mastitis in lactating goats in the Southern Rift Valley Region of Ethiopia and to find out the associated bacterial pathogens and their antimicrobial susceptibility patterns to different antimicrobial agents.

MATERIALS AND METHODS

Study Animals

The study was undertaken in Adami Tulu district situated in the Southern Rift Valley Region of Ethiopia, 167 km south of Addis Ababa (Ethiopia). In the study areas, goats are preferred animals next to cattle, and goat milk is widely consumed. The study animals were randomly selected lactating goats in the district. Out of the total goat population in the district (about 85,365 goats; according to the district's agricultural office), 17% are lactating. The predominant goat breeds in the study area are Arsi-Bale breeds, which are managed under an extensive management system by individual farmers. Farmers who own more than 5 goats keep their goats in barns while those who own less than 5 goats keep them in their own houses. Most of the farmers milk their goats in the morning once a day and few of them twice a day. They do not follow pre- and post-milking hygienic practices during milking.

Milk Sample Collection

The study involved 340 randomly selected lactating goats in the district. Physical examination including observation and palpation of the udders for symmetry and size, indurations and fibrosis, milk consistency and color change, and any visible abnormalities were recorded. Signs of inflammation, lesions on the udder/teat skin, and presence of ticks were observed. Udders and teats were cleaned and dried before sample collection. The teats were disinfected with 70% alcohol before sampling. The first few streams of milk were discarded and about 10 to 20 mL of milk were collected in sterile universal bottles. Milk samples were transported in an icebox with ice to the laboratory for microbiological analysis. Clinical mastitis was detected by gross signs of udder infection and abnormal milk whereas subclinical mastitis was recognized by apparently normal milk and positive California mastitis test (CMT) and culture results.

California Mastitis Test

Milk samples from each halve were tested for subclinical mastitis using the CMT. The results for CMT were recorded as 0, trace, 1+, 2+, and 3+.[2,14] In this study, CMT scores of 0 and trace were considered as negative while CMT scores of 1+, 2+, and 3+ were taken as indicators of subclinical mastitis.

Isolation and Identification of Bacteria

Bacteriological examination of the 680 milk samples was carried out irrespective of CMT results following standard procedures.[2,14] About 10 μL milk from each halve was inoculated onto tryptose blood agar base (BBL, Becton Dickinson, USA)

enriched with 7% defibrinated sheep blood and MacConkey agar (Oxoid, Hampshire, England) and incubated aerobically at 37°C for 24 to 48 hours. Each plate was examined for growth, morphology, and hemolytic characteristics. Identification of bacteria on primary culture was made on the basis of colony morphology, hemolytic characteristics, Gram-stain reaction, and catalase tests. Staphylococci were identified based on catalase test, growth characteristics on mannitol salt agar (Merck, Darmstadt, Germany), and tube coagulase test. Isolates presumptively identified as streptococci were characterized according to CAMP reaction, growth characteristics on Edward's media (Merck, Darmstadt, Germany), catalase test, hydrolysis of esculin and sodium hippurate, and sugar fermentation tests. Gram-negative isolates grown on MacConkey agar were identified using the oxidase reaction, catalase test, triple sugar iron (TSI) agar (Merck, Darmstadt, Germany), the "IMViC" (indole, methyl red, Voges-Proskauer, and citrate) test, and urease and sugar fermentation tests.[2,14]

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was conducted on randomly selected bacterial pathogens (n = 150) isolated during the study period to 8 antimicrobials using the Kirby-Bauer disk diffusion method.[2,15] The following antimicrobial disks (Oxoid, Basingstoke, UK) with their corresponding concentrations were used: streptomycin (Str, 10 µg), oxytetracycline (Oxt, 30 µg), erythromycin (Ery, 15 µg), gentamicin (Gen, 10 µg), chloramphenicol (Chl, 30 µg), cloxacillin (Clx, 5 µg), compound sulphonamide (Sul, 300 µg), and methicillin (Met, 5 µg). Each isolate to be tested was diluted in sterile saline solution to a 0.5 Mcfarland standard. The diluted bacterial suspension was transferred onto Müller-Hinton agar (Merck, Darmstadt, Germany) plates using sterile cotton swabs. The respective plate was seeded uniformly by rubbing the swab against the entire agar surface. Each antimicrobial impregnated disk was applied onto the surface of the inoculated plate by using sterile forceps. The plates were incubated at 37°C for 16 to 18 hours. The interpretation of the growth inhibition zones and classification of isolates as susceptible, intermediate, and resistant followed the guidelines of the National Committee for Clinical Laboratory Standards.[15] Those isolates classified as intermediate were considered as susceptible to that antimicrobial in order to avoid overestimation of resistant isolates.

RESULTS AND DISCUSSION

Clinical Mastitis

Clinical mastitis was detected in 2.4% (8/340) of the lactating goats (14 halves of 8 does). Udder injuries and tick infestations were observed in only 1.2% and 0.9% of the goats examined respectively. Of the 8 goats with clinical mastitis, 6 of them had bilateral mastitis while 2 had unilateral mastitis. The low prevalence of clinical mastitis in goats was comparable with previous reports of Ryan and Greenwood[16] who found <1% prevalence of clinical mastitis in a study conducted on 896 halves in New South Wales (Australia). Contreras et al[17] in Spain also reported prevalence of clinical mastitis ranging from 0% to 2% in a 10-year time. The low level of clinical mastitis may be partly associated with the fact that dairy goats with clinically observable mastitis are either treated or culled. A 10% prevalence of clinical mastitis had also been reported in dairy goats in Nigeria.[8] *Staphylococcus aureus* (10) was isolated from goats with clinical mastitis and was followed by *E coli* (2), *P aeuroginosa* (1), and *K pneumoniae* (1). These pathogens have been reported to be associated with clinical mastitis of dairy goats elsewhere.[8,11,18] It has been indicated that *S aureus* is one of the most commonly isolated pathogen (up to 80%) from clinical cases of goat mastitis although coagulase-negative staphylococci (CNS), *Pseudomonas* and *Pasteurella* spp. may be

involved.[19] A milk sample from a single doe with clinical mastitis yielded no bacterial growth during culture. This could be either due to earlier infection that had been overcome by the body's defense or the udder may have been infected with organisms that need special media and thus missed in the ordinary course of bacterial isolation. Furthermore, there are reports indicating that mastitis causing coliforms in 20% of the clinical cases become negative on culturing, and coliform infections are usually less than 10 days duration and are rapidly destroyed by inflammatory reactions.[2] Four goats had udder injuries, all of which had mastitis, 3 goats were infested with ticks, and 2 had mastitis. Associations between udder injuries and goat mastitis had been previously reported.[9]

California Mastitis Test

California mastitis test was conducted on 680 milk samples collected from 340 lactating goats for the presence of subclinical mastitis. Considering CMT scores of 0 and trace as negative and 1+, 2+, and 3+ as positive, 278 (40.9%) milk samples were CMT positive, while 402 (59.1%) samples were CMT negative (Table 1). On the other hand, 28 (10.1%) of the 278 CMT-positive milk samples yielded no bacterial growth while the remaining 250 (89.9%) samples were also culture positive in which diverse bacterial pathogens were identified (Table 2). It has been reported that a CMT score of 1+ or higher is a good indicator of mastitis in goats, which corresponds to a somatic cell count of greater than 0.8×10^6 cells/mL milk.[20] The authors further elaborated that using the same criteria milk samples with CMT negative and trace results could be culture positive (false negative, 20%-30%) and a number of samples with CMT scores of ≥1+ could also be culture negative (false positive, 20%-40%). Boscos et al[6] also indicated that CMT scores, particularly higher than 2+, could indicate the presence of major pathogens in goat milk. The CMT-positive and culture-negative samples could be partly explained in that the udder could be injured and is recovering from infection or the infection could be not due to a bacterial pathogen.[21] It could also be due to an organism such as mycoplasmas, which requires special media and cannot be identified in the routine bacterial isolation techniques. Of the total 402 milk samples taken as CMT negative (CMT score 0, n = 328 and trace, n = 74), 124 (30.8%) yielded bacterial growth on culture. This may be due to less pathogenic bacteria that do not induce detectable levels of somatic cell counts. This result was consistent with the reports of Ndgewa et al[22] who isolated bacteria from 22.5% of 568 CMT-negative milk samples and further indicated that these bacteria may cause latent infection or they do not stimulate detectable increase in somatic cell counts. The high prevalence of subclinical mastitis observed in our study was comparable with the findings of Anyam and Adekeye[18] from Nigeria and Moshi et al[7] from Tanzania who reported 56% and 72.8%, respectively. The level of intramammary infections in lactating goats indicates general poor management of animals prevailing in the extensive system. Absence of standard milking procedure, such as pre- and post-milking udder washing and disinfections, could also contribute to the widespread occurrence of mastitis.

Table 1. Results of California Mastitis Test (CMT) in Comparison With the Bacteriological Examinations.

	Number of Samples (%)		
CMT Score	**Examined**	**Culture Positive**	**Culture Negative**
0	328	80 (24.4)	248 (75.6)
Trace (T)	74	44 (59.5)	30 (40.5)
1+	164	143 (87.2)	21 (12.8)
2+	80	74 (92.5)	6 (7.5)
3+	34	33 (97.1)	1 (2.9)
Total	680	374 (55)	306 (45)

Table 2. Frequency Distribution of Bacteria Isolated from Clinical and Subclinical Mastitis of Goats.

Bacterial Isolate	Clinical Mastitis	Subclinical Mastitis	Total (%)
Coagulase negative staphylococci	–	39	39 (9.6)
Staphylococcus aureus	8	44	52 (12.8)
Streptococcus agalactiae	–	7	7 (1.7)
Streptococcus dysgalactiae	–	7	7 (1.7)
Other *Streptococcus* spp.	–	1	1 (<1)
Micrococcus spp.	–	19	19 (4.7)
Arcanobacterium pyogenes	–	13	13 (3.2)
Corynebacterium bovis	–	16	16 (3.9)
Corynebacterium ulcerans	–	16	16 (3.9)
Other *Corynebacterium* spp.	–	12	12 (2.9)
Bacillus spp.	–	56	56 (13.8)
Escherichia coli	2	30	32 (7.9)
Klebsiella pneumoniae	1	22	23 (5.7)
Enterobacter aerogenes	–	13	13 (3.2)
Enterobacter agglomerans	–	1	1 (<1)
Citrobacter freundii	–	4	4 (0.9)
Citrobacter diversus	–	4	4 (0.9)
Serratia marcescens	–	11	11 (2.7)
Proteus mirabilis/vulgaris	–	6	6 (1.5)
Pseudomonas auruginosa	1	31	32 (7.9)
Acinetobacter spp.	–	22	22 (5.4)
Pasteurella (*Mannheimia*) *haemolytica*	–	4	4 (0.9)
Pasteurella multocida	–	1	1 (<1)
Others	–	14	14 (3.4)

Bacterial Isolates

Out of the total 680 milk samples examined on culture, 374 (55%) yielded bacterial growth (Table 1). A total of 374 halves (177 right halves and 197) left halves from 236 goats were culture positive. About 91.7% of the milk samples (343/374) grew only one type of bacteria while 31 (8.2%) of them were mixed type. The major bacteria identified were *Staphylococcus* spp. accounting for 22.5% of the total isolates of which *S aureus* accounted for 12.8% of the total isolates followed by CNS (9.6%). Other bacterial pathogens identified include some members of the Enterobacteriaceae (*E coli*, *P aeruginosa*, *Citrobacter*, *Klebsiella*, *Acinetobacter* spp.), *Micrococcus*, *Corynebacterium*, *Bacillus*, *Streptococcus*, and *Pasteurella* spp. (Table 2). Most of these pathogens have been previously reported from mastitic goat milk samples in various research undertaken elsewhere.[4,5,9,11,22] The majority of the isolates from lactating goats in the present study have been incriminated as causes of mastitis in dairy cows[23-25] and camels[26,27] in Ethiopia. *Staphylococcus* spp. can be found widely distributed in animals, and it is a contagious pathogen that can be transmitted from doe to doe during unhygienic milking procedures.[21] Most of the clinical cases of mastitis were caused by *S aureus*, which was in agreement with the findings of Anyam and Adekeye.[18] The high prevalence of *S aureus* intramammary infection can be of veterinary and public health concern. It is one of the important zoonotic bacterial pathogens, which can also be transmitted to humans through raw milk of goats/sheep and cause food poisoning associated with enterotoxin productions.[21] Coagulase-negative staphylococci were one of the major bacterial species isolated accounting for 9.6% of the total isolates. They are contagious pathogens found on the skin of goats and human hands and can easily be transmitted during unhygienic milking procedures.[28]

Bacillus spp. accounted for 10.4% of the total isolate next to *Staphylococcus* spp. This was in agreement with findings of Anyam and Adekeye[18] in Nigeria who reported 14.7% prevalence next to *S aureus*. *Bacillus* spp. are environmental pathogens and their occurrence could be associated with poor hygienic practices in the environ-

ment where goats are kept.[29] *Streptococcus agalactiae* were identified only in 1.7% of the total isolates. The low prevalence of *Str agalactiae* was comparable with the findings of other researchers.[5,22] Kalogridou-Vassiliadou[28] showed that *Str agalactiae* and other *Streptococcus* spp. are not nearly as prevalent or economically important in dairy goats as they are in dairy cows. Coliforms and *P aeruginosa* together made up 26.6% of the total isolates. The high prevalence was consistent with the published data of Moshi et al[7] and Ameh and Tari[9] who reported 28.5% and 30% prevalence of coliforms from dairy goats respectively. The high level of occurrence could be associated with the unclean environment of goats and bedding materials. *Pasteurella* (*Mannheimia*) *haemolytica* and *P multocida* were isolated from 3 goats and 1 goat, respectively. *Pasteurella* (*Mannheimia*) *haemolytica* has been associated with clinical cases of goat mastitis.[4] The source of udder infections with this bacterium could be the noses and throats of nursing youngs.[16]

Antimicrobial Susceptibility Patterns

The majority of *S aureus* (92.5%), *Arcanobacterium pyogenes* (100%), CNS (88.2%), *Corynebacterium* (91.6%), and *Streptococcus* (77%) spp. were susceptible to the antimicrobials tested (Table 3). Different levels of antimicrobial resistance ranging from 7.5% to 83.7% were observed in the various bacterial pathogens to the different antimicrobial agents. Of the total isolates tested, a relatively high level of resistance was observed to cloxacillin (36%), methicillin (30%), streptomycin (25.3%), oxytetracycline (20.7%), erythromycin (19.3%), chloramphenicol (18.7%), compound sulphonamide (18%), and gentamicin (8.3%). Some members of the Enterobacteriaceae tested for resistance including *K pneumoniae* (83.7%), *E aerogenes* (81.3%), and *E coli* (44.3%) as well as *Bacillus* spp. (30%) exhibited a high level of resistance to methicillin, erythromycin, sulphonamide, chloramphenicol, and streptomycin. Erythromycin, streptomycin, and oxytetracycline are among the commonly used antimicrobials for the treatment of mastitis in the study areas. Our findings were consistent with the reports of Egwu et al,[11] which indicated the presence of drug resistance to bacterial pathogens, including coliforms, staphylococci spp., and streptococci spp. isolated from mastitic goats in Nigeria. Another study undertaken by Bhujbal et al[12] on the susceptibility of mastitis pathogens from goats indicated resistance to chloramphenicol (12.2%), gentamicin (21.3%), kanamycin (44%), oxytetracycline (57.6%), ampicillin (78.8%), and amoxicillin (100%). Burriel[29] reported about 75.9% drug resistance of CNS isolated from subclinical mastitis of sheep in England to tetracycline, methicillin, sulphonamide, streptomycin, and chloramphenicol. Previous studies undertaken in Ethiopia on the antimicrobial resistance of bovine mastitis pathogens[23-25] indicated the widespread occurrence and distribution of resistance to most of the antimicrobial agents which also showed resistance in the present study. This suggests the need for the prudent use of antimicrobial agents in the animal health and production sectors in the country. Results of this study has also demonstrated that both clinical and subclinical mastitis are prevalent among Ethiopian goats and the major bacterial pathogens associated were coagulase negative staphylococci, *S aureus*, *Bacillus* spp., and *Corynebacterium* spp.

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Table 3. Antimicrobial Resistance Profiles of Mastitis Pathogens to Different Antimicrobials.

Bacterial Isolate (tested)	Number (%) of Bacterial Pathogens Susceptible to								Mean (%)
	Met	Ery	Clx	Sul	Chl	Gen	Str	Oxt	
Staphylococcus aureus (52)	43 (82.7)	51 (98.1)	49 (94.2)	52 (100)	43 (82.7)	52 (100)	50 (96.2)	45 (86.1)	48.1 (92.5)
CNS (17)	15 (88.2)	16 (94.1)	12 (70.6)	12 (70.6)	16 (94.1)	17 (100)	15 (88.2)	17 (100)	15 (88.2)
Streptococcus agalactiae (7)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	1 (14.3)	0	7 (100)	5.4 (76.8)
Streptococcus dysgalactiae (7)	5 (71.4)	7 (100)	3 (42.9)	5 (71.4)	7 (100)	7 (100)	1 (14.3)	7 (100)	5.3 (75)
Corynebacterium spp. (15)	14 (93.3)	15 (100)	12 (80)	13 (86.7)	14 (93.3)	15 (100)	13 (86.7)	14 (93.3)	13.7 (91.6)
Arcanobacterium pyogenes (13)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)
Bacillus spp. (10)	8 (80)	10 (100)	0	10 (100)	10 (100)	2 (20)	10 (100)	6 (60)	7 (70)
Escherichia coli (11)	0	0	0	10 (90.9)	10 (90.9)	11 (100)	9 (81.8)	9 (81.8)	6.1 (55.7)
Klebsiella pneumoniae (10)	0	2 (20)	0	0	1 (10)	10 (100)	0	0	1.6 (16.3)
Enterobacter aerogenes (8)	0	0	0	1 (12.5)	1 (12.5)	8 (100)	1 (12.5)	1 (12.5)	1.5 (18.7)
Total (150)	105 (70)	121 (80.7)	96 (64)	123 (82)	122 (81.3)	136 (90.7)	112 (74.7)	119 (79.3)	116.7 (77.8)

Met = methicillin; Ery = erythromycin; Clx = cloxacillin; Sul = compound sulphonamide; Chl = chloramphenicol; Gen = gentamicin; Str = streptomycin; Oxt = oxytetracycline.

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