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Aantimicrobial Susceptibility of Mastitis Pathogens from Smallholder Dairy Herds in and Around Gondar, Ethiopia

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Abstract: Among the animal diseases that require antibiotic treatment in dairy herds, mastitis is the commonest one. As a consequence antimicrobial resistance of mastitis pathogens has received recent attention. The purpose of this study was to describe and compare antimicrobial susceptibility of mastitis pathogens isolated on 322 local and crossbred lactating hand milked small holder cows. The major bacteria isolated in this study were *Staphylococcus aureus* (n = 27), Coagulase-Negative Staphylococci (CNS) (n = 51), *Streptococcus agalactiae* (n = 26), *Streptococcus dysgalactiae* (n = 23), *Streptococcus uberis* (n = 11), *Micrococcus* sp. (n = 12), *Corynebacterium bovis* (n = 4), *Actinomyces pyogenes* (n = 2), *Bacillus cereus* (n = 1) *Escherichia coli* (n = 7). *Staphylococcus aureus* was found to be highly sensitive to five of the antimicrobials tested where the bacteria had shown 100% susceptibility to kanamycin and sulfisoxazole. However, Coagulase negative staphylococci had revealed different levels susceptibility for only four of the nine antimicrobials tested. *Streptococcus agalactiae* was highly susceptible to sulfisoxazole (100%), clindamycin (100%) and susceptibility to streptomycin was 50%. Similarly, all other bacteria isolated demonstrated different level of susceptibility to the tested antimicrobials. In general it was found that sulfisoxazole was the most effective antibiotic where 91.07% of the total isolates were found susceptible followed by clindamycin and kanamycin with susceptibility of (89.28%) and (88.4%), respectively. The least effective antibiotics were streptomycin (45.5%), ampicillin (49.1%). Tetracycline, erythromycin, chloramphenicol and oxacllin have susceptibility of 65.2, 59.8, 64.3 and 58.04%, respectively.

Key words: Antimicrobial susceptibility, sub clinical mastitis, bacterial isolate, dairy cattle, animal, Ethiopia

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. The keeping of dairy animals is a popular activity in many urban and peri urban areas of Ethiopia. Most of these dairy men have little knowledge of dairy husbandry and the management practices are therefore of suboptimal standards. Dairy industry in Ethiopia is still in its infancy. Little research has been done in the small holder dairy sector (Biru, 1989; Bishi, 1998; Nesru, 1999; Mungube, 2001).

Mastitis which is an inflammation of the mammary gland is among the most important diseases in the dairy animals. The occurrences, distribution and causes of mastitis in dairy cows have been reported from different countries (Radostits *et al*., 2000).

Mastitis is mostly caused by a rather limited spectrum of bacteria consisting mainly of staphylococci, streptococci and coliforms. Therefore, antimicrobials are used frequently for treatment and prevention of bovine mastitis. To successfully control mastitis and to avoid potential problems associated with bacterial resistance and treatment failure, it is important to be aware of antimicrobial resistance characteristics of mastitis pathogens.

In recent years, antimicrobial resistance has been growing concern worldwide (WHO, 1997, 2000). Acquired antimicrobial resistance in bacteria is an increasing threat in human as well as in veterinary medicine. Hence, monitoring antimicrobial susceptibility in pathogenic as well as in commensal bacteria in animals is recommended

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by OIE (Acar and Rostel, 2001). Such monitoring generates data of importance for therapeutic decisions and provides information on trends in resistance that might be cause for interventions regarding antimicrobial use. Mastitis is one of the most costly diseases for the dairy industry (Kossaibati and Esslemont, 1997) and antimicrobials are important parts of therapy of the disease. Thus, the objective of this study was to isolate the major bacterial pathogens and test their antimicrobial susceptibility of udder pathogens from dairy cows with sub clinical mastitis.

MATERIALS AND METHODS

Study area: The study was conducted in and around Gondar town, North Gondar administrative zone of Amhara National Regional State (ANRS). Gondar is located in the North-Western part of Ethiopia, 710 km North West of Addis Ababa. The study area is found at 12°40'1N longitude and 37°45'1E, latitude with an altitude range of 1802-2200 m above sea level. The soil type falls into three categories: Heavy black clay soil, loam brown and red soil and sandy loam soil. The ranges of maximum and minimum temperature vary between 22-30.7 and 12.3-17.1°C, respectively. The region receives a bimodal rainfall, the average annual precipitation rate being 1000 mm. The short rains occur during the months March, April and May while the long rains extend from June through September (MOA, 2004).

Study animals and husbandry practices: The study population comprises of approximately 322 crossbred and lactating Zebu cows in and around Gondar town managed under semi-intensive and extensive production system. In the semi-intensive production system, the animals are mainly composed of crossbred cattle. They are kept indoors and graze in the field occasionally. They are supplemented with concentrates in addition to hay. On the other hand, the extensive production system that consists of local breeds that depend for feed on grazing at the field with minor supplementation in the evening when they come back home. Local dairy cows are managed under traditional and extensive husbandry systems. The animals are relatively smaller in size and have small udder and short teats. The average daily milk production from individual cows was relatively low (4-5 L). Crossbred dairy cows are often managed under a small-scale, semi-intensive management system. They are often provided with some supplementary diet in addition to the natural pasture and crop by products and are maintained usually in separate stalls a short distance from each other in a house. This type of dairy husbandry system is increasingly becoming an important source of milk supplies to households and a means of income generation in urban and peri-urban areas of Gondar. Manure removal is generally made on a daily basis. Although, milking is done by hand, pre-milking and post-milking hygienic procedures such as udder washing and drying are not practiced. Cows are dried off at late-lactation abruptly.

Study design and sampling procedure: A cross-sectional type of study was carried out. Sample size was determined at 95% confidence interval, 5% precision and from previous studies in similar study area (Workineh *et al*., 2002) with an expected prevalence of 30%. The sample size was determined by using the formula for simple random sampling (Thrusfield, 1995). Households selected was determined by dividing sample size with herd size in this case 322/2 = 161. Hence, these study 161 households were included.

Preparing udders and teats: Udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with cotton or gauze sponge moistened (but not completely wet) with 70% ethyl alcohol. Recontamination of teats during scrubbing was avoided, the teats on the far side of the udder first then those on the near side. A separate pledged or sponge was used for each teat. Scrubbing was continued until a new surface of the cotton or sponge remains clean.

Collection of milk samples: Procedures for collecting milk sample were according to (Schalm *et al*., 1971; Sears *et al*., 1993; Quinn *et al*., 1994). Strict aseptic procedures were used when collecting milk samples in order to prevent contamination with the many microorganisms present on the skin of cow's flanks, udder and teats on the hands of the sampler and in the barn environment. Teats towards sample collection were taken first and then the far ones. The first 3-4 streams of milk were discarded. The collecting vial was as near horizontal as possible and by turning the teat to a near horizontal position, 15 mL of milk was collected into the vial.

Time of sample collection: Samples for culture were collected before milking that was most convenient under the management conditions of the individual herd.

Handling and storing samples: After collection, samples for cultures were placed in racks for ease of handling and held in an icebox, properly packed and kept cold. They were processed as soon as possible.

California Mastitis Test (CMT): The California mastitis test was carried out as screening test for selections of samples for culture following the method described by Schalm *et al.* (1971) and Quinn *et al.* (1994). A squirt of milk about 2 mL from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in a horizontal plane for 15 sec. The reaction was interpreted according to Schalm *et al.* (1971) and Quinn *et al.* (1994).

Bacterial isolation: In the milk samples that had been refrigerated, dispersion of bacteria and fat were accomplished by warming the samples at room temperature for about an hour and then mixed by shaking. Bacteriological examination of the milk was carried out following standard procedures of Carter (1984), Sears *et al.* (1993) and Quinn *et al.* (1994). One standard loop (0.01 mL) of milk sample was streaked on 7% blood agar. The inoculated plate then was incubated aerobically at 37°C for about 24, 48 and up to 72 h to rule out slow growing microorganisms. For primary identification, colony size, shape, color, hemolytic characteristics, grams reaction and catalase production were used. This was conducted at Gondar University Microbiology Laboratory, Gondar. Suspected colonies were isolated onto blood agar plates for further investigation for confirmation biochemical tests were used after sub culturing isolated distinct colonies onto a nutrient agar.

Antibiotic susceptibility test: The antimicrobial resistance patterns of the isolates were determined using the Kirby Bauer disk diffusion technique. The disks were impregnated with the following antibiotics: tetracycline, erythromycin, kanamycin, chloramphenicol, streptomycin, ampicillin, sulfisoxazole, oxacillin and clindamycin.

Discs were stored under refrigeration to ensure maintenance of their potency. Well-isolated colonies of the same morphologic type were inoculated into 5 mL of a nutrient broth incubated at 37°C for 5 h until a visible turbidity appeared. The turbidity was compared to the 0.5 Mc far land standards. Mueller Hinton agar was used as plating medium. About 15 min after the plates were inoculated, antibiotic impregnated discs were applied to the surface of the inoculated plates with sterile forceps. All discs were gently pressed down onto the agar with forceps to ensure complete contact with the agar surface; the plates were inverted and then aerobically incubated for 18 h at 37°C. The diameters of the zones of complete inhibition were measured to the nearest whole millimeter using a ruler. Zones of inhibition for individual antimicrobial agents were translated into susceptible, intermediate and resistant categories by referring the recommended NCCLS interpretative standards.

Retrospective data were compiled on the type of antibiotics used to treat mastitis and other infectious diseases in the region. Specifically the antibiotics used to treat clinical mastitis cases were gathered from clinical casebook records.

Antibiotics commonly used in mastitis treatment after the isolation of etiological agents were made. Interpretation of the results was made according to inhibition diameter. Bacteria tested were classified in the following categories of sensitivity: sensitive, intermediate sensitive or resistant. In that study researchers used the following antibiotics: tetracycline, chloramphenicol, streptomycin, oxacillin, ampicillin, sulfisoxazole, erythromycin, kanamycin and clindamycin.

Statistical analysis: Data on distribution of bacterial isolates and antimicrobial susceptibility were described as frequencies and percentages. Comparisons of bacterial isolates and antimicrobial susceptibilities between before and after were performed by Fisher's exact, χ^2-tests.

The significant levels were defined at $p<0.05$. The proportion of bacteria resistant to each antibiotic was calculated. Also, the proportions of bacteria resistant to at least one of the 9 tested antibiotics and resistant to two or more antibiotics were calculated. The associations between dependent and independent variables were tested by logistic regression.

RESULTS AND DISCUSSION

Bacterial isolation: The major bacteria isolated in this study were *Staphylococcus aureus* (16.5%), *Streptococcus agalactiae* (15.9%), *Streptococcus dysgalactiae* (14.0%), *Streptococcus uberis* (6.7%), *Escherichia coli* (4.3%) and *Actinomyces pyogenes* (1.2%). Under minor pathogens CNS accounted for (31.1%), *Micrococcus* species (7.3%). *Corynebacterium bovis* (2.4%) and *Bacillus cereus* (0.6%). The prevalence rates of various bacterial pathogens in local zebu and crossbred lactating cows are shown in Table 1.

The majority of the isolates in this study were from cases of sub clinical mastitis. Out of 12 quarters affected clinically, bacteria were isolated from 7 quarters only; the remaining 5 yielded no bacteria. Isolates from clinical cases were *Escherichia coli*, CNS and *Streptococcus agalactiae* (Table 2).

Antibiotic susceptibility results: The result of bacteriological analysis are shown in Table 3 from a total of 164 isolations were 27 *S. aureus*, 51 CNS, 26 *Str. agalactiae*, 23 *Str. dysgalactiae* 11 *Str. uberis*, 12 *Micrococcus* species, 4 *C. bovis*, 7 *E. coli*, 2 *A. pyogenes* and 1 *B. cereus* were isolated. Antimicrobial susceptibility test was done on 112 isolates. The distribution and percentage of isolates tested for antibiotic susceptibility are shown (Table 3).

Staphylococcus aureus was highly sensitive to kanamycin (100%), sulfisoxazole (100%), clindamycin (88.9%), chloramphenicol (81.5%), tetracycline (70.4%) and was resistant to ampicillin (only 18.5% susceptibility). In this study, sulfisoxazole and chloramphenicol were most effective on *S. aureus* isolates. Coagulase negative staphylococci was more susceptible to sulfisoxazole (88%), kanamycin (84%) tetracycline (80%) and clindamycin (76%) however CNS was resistant to ampicillin (44%), chloramphenicol (44%), streptomycin (44%) and oxacillin (40%). *Streptococcus agalactiae* was highly susceptible to sulfisoxazole (100%), clindamycin (100%) and susceptibility to streptomycin was 50%. *Streptococcus dysgalactiae* showed susceptibility to clindamycin (100%), ampicillin and kanamycin (80%) and was resistant to streptomycin (100% resistant). *Streptococcus uberis* was most sensitive to chloramphenicol (100%), sulfisoxazole (100%), oxacillin (100%), clindamycin (100%), kanamycin (85.7%) and least responsive against erythromycin (20% susceptibility). *Micrococcus* species was highly sensitive to erythromycin (100%), kanamycin (100%) and clindamycin (100%) but poorly susceptible to streptomycin and chloramphenicol (50%).

Escherichia coli was found highly susceptible to chloramphenicol (100%), kanamycin (100%) and sulfisoxazole (100%) however was highly resistant to erythromycin (only 20% susceptibility) to ampicillin and tetracycline (40%). *Corynebacterium bovis* was found highly susceptible to tetracycline (100%), kanamycin (100%) and sulfisoxazole (100%) but resistant to oxacillin (25%). *Actinomyces pyogenes* was found highly susceptible to sulfisoxazole (100%) but was 100% resistant to tetracycline, chloramphenicol and oxacillin. *Bacillus cereus* was susceptible to ampicillin (100%), tetracycline (100%), kanamycin (100%), sulfisoxazole (100%) and clindamycin (100%). However, these 100% bacteria were resistant to erythromycin, chloramphenicol and oxacillin.

Table 1: Frequency of bacteria isolated from mastitic milk in cross breed and local zebu lactating cows

	Local breed		Cross breed		
Isolates	Frequency	(%)	Frequency	(%)	Total
Staphylococcus aureus	3	33.33	24	15.48	27
Streptococcus agalactiae	1	11.11	25	16.13	26
Streptococcusdysgalactiae	1	11.11	22	14.19	23
Streptococcus uberis	-	-	11	7.08	11
Actinomyces pyogens	-	-	2	1.29	2
Escherichia coli	-	-	7	4.52	7
Coagulase negative staphylococci	2	22.22	49	32.26	51
Micrococcus species	2	22.22	10	6.45	12
Corynebacterium bovis	-	-	4	2.58	4
Bacillus cereus	-	-	1	0.66	1
Total	9	100.00	155	100.00	164

Table 2: Frequency distribution of bacteria isolated from mastitic milk

Isolate	Frequency	Percentage
Staphylococcus aureus	27	16.5
Coagulase negative staphylococci	51	31.1
Streptococcus agalactiae	26	15.9
Streprococcus dysgalactiae	23	14.0
Streptococcus uberis	11	6.7
Micrococcus sp.	12	7.3
Corynebacterium bovis	4	2.4
Actinomyces pyogenes	2	1.2
Bacillus cereus	1	0.6
Escherichia coli	7	4.3
Total	164	100.0
Mixed growth	16	-
No growth	1069	-
Total	1249	-

Table 3: Results of antibiotic susceptibility tests on bacteria isolated from milk samples obtained from cows with mastitis

Bacterial isolates	N	TET	CMP	S	Oxa	Amp	Su	ERY	K	CI
S. aureus	27	19 (70.4)	22 (81.5)	14 (51.8)	22 (81.5)	5 (18.5)	27 (100.00)	14 (51.8)	27 (100.00)	24 (88.9)
CNS	25	20 (80.0)	11 (44.0)	11 (44.0)	10 (40.0)	11 (44.0)	22 (88.00)	15 (60.0)	21 (84.00)	19 (76.0)
Str. agalactiae	20	12 (60.0)	16 (80.0)	10 (50.0)	15 (75.0)	12 (60.0)	20 (100.00)	16 (80.0)	16 (80.00)	20 (100.0)
Str. dysgalactiae	15	6 (40.0)	6 (40.0)	0 (0.0)	4 (26.7)	12 (80.0)	10 (66.70)	9 (60.0)	12 (80.00)	15 (100.0)
Str. uberis	7	4 (57.1)	7 (100.0)	4 (57.1)	7 (100.0)	4 (57.1)	7 (100.00)	3 (20.0)	6 (85.70)	7 (100.0)
Micrococcus sp.	6	5 (83.3)	3 (50.0)	3 (50.0)	4 (66.7)	4 (66.7)	4 (66.70)	6 (100.0)	6 (100.00)	6 (100.0)
E. coli	5	2 (40.0)	5 (100.0)	4 (80.0)	2 (40.0)	2 (40.0)	5 (100.00)	1 (20.0)	5 (100.00)	4 (80.0)
C. bovis	4	4 (100.0)	2 (50.0)	3 (75.0)	1 (25.0)	3 (75.0)	4 (100.00)	2 (20.0)	4 (100.00)	3 (75.0)
A. pyogenes	2	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	2 (100.00)	1 (50.0)	1 (50.00)	1 (50.0)
B. cereus	1	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (50.0)	1 (100.00)	0 (0.0)	1 (100.00)	1 (100.0)
Total	112	73 (65.2)	72 (64.3)	51 (45.5)	65 (58.04)	55 (49.1)	102 (91.07)	67 (59.8)	99 (88.39)	100 (89.2)

Values in brackets indicate antimicrobial susceptibility in percentage, TET = Tetracycline, CMP = Chloramphenicol, S = Streptomycin, Oxa = Oxacillin, Amp = Ampicillin, Su = Sulfisoxazole, ERY = Erythromycin, K = Kanamcin, CL = Clindamycin, n: Total number of isolates tested

Table 4: Mastitis cases and type of drugs used for therapy in Gondar veterinary clinic

Types of drugs	No. of mastitis cases treated	Percentage
Pen strep	26	30.95
Ox tetracycline	14	16.67
Intra mammary infusion	26	30.95
Mastitis injector	15	17.86
Procaine penicillin	3	3.57
Total	84	100.00

In general it was found that for the bacterial isolates tested for antimicrobial susceptibility, sulfisoxazole was the most effective antibiotic where 91.07% of the total isolates were found susceptible followed by clindamycin and kanamycin with susceptibility of 89.28% and 88.4%, respectively. The least effective antibiotics were streptomycin (45.5%), ampicillin (49.1%). Tetracycline, erythromycin, chloramphenicol and oxacllin have susceptibility of 65.2, 59.8, 64.3 and 58.04%, respectively.

Frequently used antibiotics to treat case of mastitis: A 1 year retrospective data (2006) obtained from clinical records in the study area showed that the types of antibiotics used to treat mastitis were Pen Strep (penicillin and streptomycin combinations), oxytetracycline and intramammary infusion, procaine penicillin, mastitis injector (Table 4).

Bacterial isolation: In this study, S*taphylococcus aureus* and CNS were the major mastitis inducing pathogens detected. CNS was the predominant pathogens involved constituting (31.1%) of all isolates. This finding was at variance from earlier investigations in other regions in Ethiopia. Bishi (1998) and Hussien *et al.* (1997) reported 54 and 42% isolation rates of *S. aureus* and CNS, respectively. In Denmark, out of 4645 quarter milk samples examined to determine the distribution of bacterial species in bovine mastitic milk, CNS was the second predominant isolate next to *S. aureus* (Nickerson, 1987). Workineh *et al.* (2002) reported isolation of CNS at a rate of 2.5% lower than the present study. The high isolation rate of CNS in this study could be associated with lowered resistance of the cow due to teat injury. Staphylococci typically colonize a broken skin and hence the risk of colonization and subsequent transfer into the udder increases. Actually, the effect of tick and/lesion on the occurrence of mastitis in this study was not significant.

In the Coagulase negative staphylococci, the primary source of these organisms appears in skin contamination tending to be associated with a lack of teat dipping. Some contribution to the number of these organisms can occur from intra mammary infections but skin flora appears to be the major factor. The uses of post milking teat dipping and routine dry cow therapy markedly reduce the prevalence of Coagulase negative staphylococci in dairy herds. Cure rate for lactation therapy are usually poor but dry cow therapy will cure >80% of existing infections. Pre dipping with an effective germicide will control new infections. The isolation rate of *S. aureus* (16.5%) in this study was the next to CNS and closely comparable with the findings of Bishi (1998) and Hussien *et al.* (1997) reported 9 and 10.69% prevalence in Addis Ababa, respectively. However, the present finding was lower than that of Workineh *et al.* (2002), Dego and Tareke (2003) where *S. aureus* accounted for 39.2 and 40.5% of the isolates at Addis Ababa and Southern Ethiopia, respectively. The relatively high prevalence of *S. aureus* in this study could be associated with lack of effective udder washing and drying, post milking teat dip and drying, inter cow hand washing and disinfection in the milking routine of the area, contamination of milkers hands, low culling rate of chronically infected cows and limited knowledge of dairy holders on segregation as a control option has been reported to quickly lead to spread of mastitis and *Str. uberis* (6.7%) were isolated.

This finding was higher than the finding of Dego and Tareke (2003) who reported isolation rates of 13.1% *Str. agalactiae*, 5.6% *Str. dysgalactiae streptococci* species were also among the dominant (36.6%) bacterial population as mastitis pathogens in and around Gondar milk shed. *Str. agalactiae* (15.9%), *Str. dysgalactiae* (14.0%) and *Str. uberis* 5.1%.

The spread of *Streptococcus agalactiae* like *Staphylococcus aureus* occurs during milking. Excellent milking hygiene and the use of an effective teat dip could reduce transmission from cow to cow. In this study, contagious pathogens showed greater frequency than others (*S. aureus* being the most common). Poor milking and management practices and hired milkers who milk in more than one farm could explain this. The explanation given for *S. aureus* could also apply for *Str. agalactiae* and *Str. dysgalactiae*.

In this study, environmental pathogens for *Str. uberis*, *Str. dysgalactiae* and *Escherichia coli* were isolated. There is a common understanding that with increasing herd size, manure disposal and sanitation problems leads to build up of bacterial population (Coli forms and environmental Streptococci) in the cow's immediate environment. In this study, the average herd size was two lactating cows. The number of hours dairy cows kept indoor is also a factor that will increase the possibility of contact of teats with the environmental pathogens.

Environmental mastitis pathogens were found to be the most frequent isolates from clinical quarter cases in a

random sample of dairy herds in Southern Netherlands (Miltenburg *et al*., 1996). In the milking barn, the floor of the udder and teats should be as clean and dry as possible prior to milking. This is extremely important because wet milking of cows increases the incidence of infections caused by environmental Streptococci. Generally, the key to control is through good sanitation.

Generally, in small holder management system as in this study that cows were allowed to graze for longer hours a day on the pasture land and supplemented with concentrate and hay when they return home late in the after noon. This might minimize their stay indoor and hence minimal exposure rate to environmental pathogens. The low prevalence of clinical mastitis in this study could be associated with this management system.

Antibiotic susceptibility test: The present study was under taken to determine the resistance pattern of bovine mastitis causal bacteria to commonly used antimicrobials in the study area to provide information to concerned animal health professionals. The antimicrobials response rate may be qualified as poor when it cures ≤25% and said favorable when the response rate attains 75% or above. The selection of the types of antimicrobial agents was made based on clinical considerations including frequent use of the drug in the study area and availability. Tetracycline, pen strep, intramammary infusion, mastitis injector was commonly used antimicrobials for the treatment of mastitis in the study area. The selection of the types of antimicrobial agents was made based on clinical considerations including frequent use of the drug in the study area and availability. Tetracycline, pen strep, intramammary infusion, mastitis injector was commonly used antimicrobials for the treatment of mastitis in the study area.

The poor inhibitory effect of ampicillin against *Staphylococcus aureus* strains observed in this study is in agreement with what was reported by Mackie *et al*. (1988). The latter reported that the *S. aureus* isolates sensitivity to ampicillin was 19 and 17% from clinical and subclinical mastitis, respectively for the year 1986 in Northern Ireland.

In this study, *S. aureus* isolates were most susceptible to kanamycin, sulfisoxazole, clindamycin, oxacillin, chloramphenicol, erythromycin, streptomycin and tetracycline. *S. aureus* showed resistance to ampicillin. Bishi (1998) obtained comparable results where erythromycin and oxacillin were effective on *S. aureus* where as streptomycin was less effective where only 18% of the total isolates were susceptible. In the present study, unlike (Bishi, 1998) tetracycline and chloramphenicol were effective only in 12 and 18% of the isolates. *E. coli* was highly resistant to erythromycin and highly susceptible to chloramphenicol and kanamycin. This was lower than the report given by Erskine (2001).

In general sulfisoxazole, clindamycin and kanamycin were showed very good efficacy; tetracycline, chloramphenicol, erythromycin and oxacillin showed moderate efficacy whereas streptomycin and ampicillin showed poor efficacy in almost all isolates.

Each herd should have a treatment protocol designed by a consulting veterinarian. A limited selection of drugs, chosen according to the results from bacteriological diagnoses and sensitivity tests should be used for treatment. Treating sub clinical mastitis during lactation with antimicrobial drugs should be avoided due to substantial economic losses arising through milk having to be discarded and unsatisfactory cure rates. Treatment of clinical mastitis should always also include monitoring of therapy results.

The present study demonstrated the existence of alarming level of resistance of frequently isolated mastitis bacteria to commonly used antimicrobial agents from prolonged and indiscriminate usage. It is therefore, very important to implement a systematic application of an *in vitro* antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra mammary infections. Mean while, due to limited sample size tested in the present study additional studies involving larger sample size and dairy herds will have greater use to formulate guidelines with regard to the choice and use of antibiotics in both the treatment and prevention of intra mammary infections.

CONCLUSION

Ampicillin and streptomycin showed poor efficacy in the majority of the isolates sulfisoxazole, clindamycin and kanamycin were showed very good efficacy therefore could be the drug of choice for the study area. Each herd should have a treatment protocol designed by a consulting veterinarian. A limited selection of drugs, chosen according to the results from bacteriological diagnoses and sensitivity. Awareness should also be created among smallholder farmers about the economic impacts and benefits of controlling mastitis. 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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