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Microbiological study on bacterial causes of bovine mastitis and its antibiotics suscebtibility patterns in East Showa Zone, Akaki District, Ethiopia

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The study was conducted during September, 2008 to 2009 in East Showa Zone, Akaki district to determine the prevalence of mastitis, identify risk factors, isolate and identify major bacterial causes and conduct *in-vitro* antimicrobial susceptibility test. Forty six (46) small holder dairy farms and 200 dairy cows were selected by one stage cluster sampling. Questionnaire survey, farm inspection and clinical examination of cows were used to collect data. Milk samples which were positive on california mastitis test (CMT) was collected aseptically using sampling bottles and transported to veterinary microbiology laboratory at Addis Ababa University, Faculty of veterinary medicine for bacteriological study. The prevalence of clinical mastitis at herd, cow and quarter level accounted 17.3, 3.0 and 1.2%, respectively whereas 60.8% herd, 25.0% cow and 12.7% at quarter level during subclinical mastitis. Major bacterial isolates in subclinical mastitis include *Staphylococcus aureus* (38.9%), coagulase negative *Staphylococcus* (23.4%), *Bacillus* spp. (10.4%), *Escherichia coli* (7.8%), *Streptococcus agalactiae* (6.5%), *Streptococcus dysgalactiae* (1.3%), *Staphylococcus uberis* (1.3%) and *Staphylococcus intermidius* (5.2%). Likewise during clinical mastitis, *S. agalactiae* and *S. aureus* accounted (33.3%) and (22.2%), respectively. Univariate logistic regression indicated that stage of lactation, parity number, teat lesion and milking mastitic cow at last had significant effect ($p < 0.05$) on prevalence of mastitis. The *in vivo* antimicrobial sensitivity tests showed that gentamicin, kanamycin, chloramphenicol and vancomycin were the most effective antibiotics, followed by streptomycin and penicillin; bacitracin, polymxin and amoxicillin were least effective drugs.

Key words: Antibiotics Sensitivity test, mastitis, prevalence, risk factors.

INTRODUCTION

Dairy production is a biologically efficient system that converts large quantities of roughage, which is the most abundant feed in the tropics into milk and meat (Bradley, 2002). Milk is a very nutritional food that is rich incarbohydrate, proteins, fats, vitamins and minerals. However, milk can be associated with health risk to consumers, which is linked to presence of zoonotic pathogens and anti-microbial drug residues. The quality

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of milk may be lowered by a number of factors such as milk adulteration, contamination during and after milking and presence of udder infection (Esron et al., 2005).

Milk serves as an excellent medium for certain microorganisms. Particularly, bacterial pathogens whose multiplication depends mainly on temperature compete with other microorganisms and their metabolic products with regards to their disease producing capacity. These pathogens depend upon the initial load of infection in the milk and on the subsequent dilution, processing, time lapse before the milk is consumed and other factors (Wellenberg et al., 2002). Pathogenic organisms in milk are derived from different sources such as the cow itself, human hands or the environment. These organisms can be excreted from the udder through the milk or may originate from the skin and mucous membranes of the animal or milker, resulting in the contamination of milk and utensils (Bradley, 2002).

Mastitis is an inflammation of mammary gland. It is a highly prevalent problem in dairy cattle and is one of the most important threats affecting the world's dairy industry. Although it may be caused by chemical or physical agents, the causes are almost entirely infectious and mostly are bacterial infections. At least 137 biological infectious agents causing bovine mastitis are known to date and in large animals the commonest pathogens are *Staphylococcus* species, *Streptococcus* species and *Coliforms.* It may be associated with many other microorganisms including *Actinomyces pyogens, Pseudomonas aeruginosa, Nocardia asteroids, Clostridium perfringens* and others like *Mycobacterium*, *Mycoplasma*, *Pastuerella* and *Prototheca* and *Yeasts* species (Radostits et al., 2000)

Bovine mastitis can be clinical and the clinical signs vary with the severity of the disease which includes pain, heat and swelling of the affected quarter. Half of the gland shows abnormality of milk either as clots or flakes and wateriness of the milk. Sub-clinical mastitis manifests with production losses and lowered milk quality. Both forms of mastitis produce significant economic losses due to rejection of milk for consumption, degraded milk quality and early culling of cows (Mifflin, 2004).

The economic impacts of bovine mastitis and intra-mammary infection have lead to the development of various therapeutic strategies to control them. Many drugs belonging to various therapeutic classes have been assessed (Koivula et al., 2005). The production of high quality milk is realistic and a meaningful goal for all aspects of dairy industry and the primary motivator for establishing a mastitis control program in dairy herd. Herds that have undergone successful comprehensive mastitis control program also need to develop strategies to control infection with environmental organism and need to use an effective monitoring system for new infection (Smith et al., 1985).

In Ethiopia, the disease is inadequately investigated and information related to its cause, magnitude of distribution and the risk factor is scanty. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effect. The conventional drugs used for treatment of mastitis are of limited values in most of the woredas (districts), and due to this and other factors causative agents have showed variable degree of resistance. Some of the bacterias like *S. aureus*, *Streptococcus* species and some other pathogens have already developed resistance to many antibiotics (Kerro, 1997). The present study attempted to determine the prevalence of bovine mastitis, identify risk factors, isolate and identify the bacterial causes of bovine mastitis and conduct *in vitro* antimicrobial susceptibility test on those isolated pathogens.

MATERIALS AND METHODS

Study area

Akaki district is located 35km away from Addis Ababa at 9°-10°24' North latitude and 37°56'-40°35' East longitude with an altitude range of 1500-3100 meter above sea level. Its annual temperature ranges from 15°C-27°C. The mean annual rainfall of the district is 800-900 mm and the short rain occurs during February, March and April and the long rain extends from June up to August (Unpublished data of 2010/11). The report also shows that of all the domestic animals raised in the District, cattle population takes the first rank with 91,040, followed by 39,055 goats, 39,048 sheep, 22,676 donkeys, 6,136 horses, and 2,015 mules.

Study population and study design

Smallholder dairy farms in Akaki districts and dairy cattle owned by them represented the study population. The study was a cross-sectional type with some retrospective surveys and it was conducted from September, 2008 to May, 2009 on smallholder dairy farms. The data collection methods were based on structured questionnaire survey, California mastitis test (CMT), milk sample collection, bacteriological culture and *in vivo* antimicrobial susceptibility tests.

Sample size determination and sampling procedure

The sample size was determined using simple cluster type of sampling where farms were sampling units in which all dairy cattle in the farm were included in the study. The sample size was determined based on the formula recommended by (Thrusfield, 2005).

$$g = \frac{1.962}{nvc + Pexp\,(1-Pexp)\,nd^2}$$

Where g = number of clusters to be sampled, Pexp = expected prevalence, d = desired absolute precision, n = number of animals per cluster and vc = between cluster variance.

With 95% confidence level, 5% precision, an average herd size of 5

animals and 60% prevalence from previous studies (Workneh et al., 2002), forty six (46) clusters samples were selected randomly in Akaki Woreda. Thus, two hundred (200) dairy cattle were included in the study from Akaki districts.

Data collection

Structured questionnaire survey was conducted to collect data on potential risk factors for the occurrences of mastitis. The animals level factors considered in study were parity numbers, herd size, stage of lactation and presence of teat lesion. The farm level factors were housing, farm hygiene, and barn floor status, milking hygiene and milking sequence. The questionnaire survey format was structured and it was pre-tested and administered to owners of the animal.

Clinical examination of cows

The clinical inspection of the udder was done in the following way. The udder of selected animals was first examined visually and then by palpation to detect fibrosis, inflammatory swelling, visible injury, tick infestation, atrophy of tissue and swelling of supra mammary lymph nodes. The size and consistency of mammary gland were inspected for presence of any abnormalities such as disproportional symmetry, swelling, firmness and blindness.

California mastitis test

The CMT was carried out as screening test for sub-clinical and clinical mastitis and for selection of samples for culture. 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 commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in horizontal plane for 5 s. The reaction was interpreted based on the thickness of the gel formed by CMT reagent and milk mixture, and the test result were scored as negative (0), trace (T), + (weak positive), ++ (distinctive positive) and +++ (strong positive) according to (Quinn et al., 2002). Quarters with CMT score of (+) or above were judged as positive. Cows were considered positive when at least one of the quarters becomes positive for CMT and a herd was considered positive, when at least one cow in the herd was tested positive with CMT.

Milk sample collection

Milk samples were collected according the procedure recommended by Quinn et al. (2002). Strict aseptic procedures were followed while collecting milk samples in order to prevent contamination with microorganisms present on the skin of cow's flasks, udder and teats, on the hands of the samplers and in the barn environment. The teats were washed with soap and water and dried with towel, 70% ethyl alcohol was also applied before sample collection. Sterile universal bottle with tighten fitting caps were used. The universal bottle was marked or labeled with permanent marker before sampling. First few streams of milk were removed and discarded to reduce the number of contamination bacteria in the teat canal. To reduce contamination of the teat ends during sample collection, the near teats were sampled first then (Wellenberg et al., 2002) followed by the far ones. Universal bottle was held as horizontal as possible to the teat and 15 ml of milk sample was collected into the universal bottle. After samples were collected, they were properly packed and stored in an icebox and transported to the Microbiology Laboratory of Faculty of Veterinary Medicine of Addis Ababa University (FVM-AAU).

Bacterial isolation and identification

Bacterial isolation and identification was conducted at the Microbiology Laboratory of FVM-AAU. Bacteriological examination of the milk was carried out using the standard procedure (Quinn et al., 2002). One loop (0.01 ml) of milk sample was streaked on 5% blood agar. Inoculated plates were incubated aerobically at 37°C and examined for growth at 24 to 48 h. The isolated micro-organisms were analyzed by their colony characteristics. From culture positive plates, typical colonies were subjected to Gram's stain to see the staining properties and cellular morphology of the bacteria. Pure cultures of a single colony from the blood agar were transferred into nutrient and blood agar plates for further bio-chemical examinations such as coagulase, catalase, DNase, triple sugar iron (TSI) and indole, methyl red, Voges-Proskauer, and citrate (IMViC) tests.

Antimicrobial sensitivity test

The antimicrobial susceptibility pattern of the bacterial isolates was determined using the Kirby-Bauer-disk diffusion method (Quinn et al., 1994). The disks were impregnated with the following antibiotics: kanamycin (K 30), streptomycin (S 10), penicillin 10 units (P 10), amoxicillin (Aml 2), gentamicin (CN 10), chloramphenicol (C 30), polymyxin (PB 300), bacitracin (B 10) and vancomycin (VA 30). Disks were stored under refrigeration to ensure maintenance of their potency. Well isolated bacterial colonies of the same morphologic type were inoculated into 5 ml of a Tryptophan soy broth and incubated at 37°C for 8 h until a visible turbidity was compared to the 0.5 McFarland standards. Mueller Hinton agar for less fastidious bacterial isolates and 5% sheep blood agar for *Streptococcus* species isolates were used as planting medium. Fifteen minutes after the plates were inoculated antibiotic impregnated disks were applied to the surface of the inoculated plates with sterile forceps. All disks 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 zone inhibition were measured to the nearest whole millimeter using the transparent ruler. Zones of inhibition for individual antimicrobial agents were translated into susceptible, intermediate and resistant categories by referring the recommended interpretative standards (NCCLS, 2000).

Data storage and analysis

Data collected through questionnaire survey, farm inspection, animal examination, bacterial isolation and identification and antibiotic susceptibility test were entered into the data base management software, Microsoft-Excel computer program (Version 6.0., 2000). Descriptive statistics was estimated using SPSS for windows (release 15.0, 2006). Analyses of associations between the prevalence of subclinical mastitis at quarter, cow and herd level, with risk factors, were estimated by univariate and multivariate logistic regression of STATA (Version 10, 2007).

RESULTS

The current study revealed that out of 200 dairy cattle an

overall prevalence of sub clinical and clinical mastitis based on CMT and culture accounted 25% (n = 50) and 3% (n = 6), respectively.

Prevalence of clinical mastitis

On the bases of CMT and clinical observation 17.3% (n = 8) herds, 3.0% (n = 6) cows and 1.2% (n = 10) quarters had clinical mastitis. Most of the quarters were affected by acute mastitis which is characterized by swelling of udder and change of milk content. Milk samples were aseptically collected for CMT, positive dairy cattle were inoculated into culture media and enabled to isolate bacteria from 17.3% (n = 8) herds, 3.0% (n = 6) cows and 1.2% (n = 10) quarters (Table 1).

Prevalence and risk factors affecting sub clinical mastitis

The prevalence of sub clinical mastitis was determined by CMT and microbiological cultures as presented in Table 1. Of 200 cows examined, sub-clinical mastitis accounted 60.8% (n = 28) herd, 25% (n = 50) cow and 12.7% (n = 101) quarters level. Bacterial culture for CMT positive dairy cattle indicated prevalence of 50% (n = 23) at herd, 24% (n = 48) at cow and 9.1% (n = 91) at quarter level.

The prevalence of subclinical mastitis at cow level was significantly affected ($p < 0.05$) by stage of lactation, parity number and presence of teat lesion. The prevalence was significantly higher in cows at the end of lactation (86.1%), with high parity number (55.7%) and teat lesion (80%). When those factors with p-value less than 0.25 were fitted in the multivariate model, only stage of lactation had significant effect on cow level prevalence ($p < 0.05$). Cows at the end of lactation were more affected by subclinical mastitis than others (OR = 30.33) (Table 2).

Regarding herd level prevalence, only the practice of milking mastitic cows last had significant effect ($p < 0.05$). Almost seventy seven percent (77.6%) of dairy farms which were not practicing udder washing before milking were prone to mastitis, whereas only 68.1% of the dairy farms which were practicing udder washing were infected. Among selected dairy farms, only one farm which practice hand washing before milking was infected with mastitis (71.4%). Risk factors with p-value less than 0.25 were fitted in a multivariate model and only the practices of milking mastitic cow last had significant effect on herd level prevalence of subclinical mastitis (Table 2).

Bacterial isolates

From the total isolates, the major contagious pathogens were *S. aureus* and *S.agalactaie* and accounted for 37.2 and 9.3%, respectively. The most important pathogens isolated from clinical cases as indicated in Table 3 were *S. agalactaie* 3 (33.3%), *S. aureus* 2 (22.2%) and coagulase-negative *Staphylococci* (CNS) 2 (22.2%). In the case of subclinical mastitis, *S. aureus* 30 (38.9%), CNS 18 (23.4%), and *Bacillus* species 8 (10.4%) were the most frequently isolated pathogens. The major environmental pathogens isolated were *E. coli* 6 (7.8%), *S. uberis* (1.2%), *S. dysgalactaie* 1 (1.3%) and *A. pyogense* 2 (2.6%). Other minor isolated pathogens included *Bacillus* species, *S. intermidius*, *Micrococcus*, *Corynebacterium bovis and Coagulase negative staphylococcus* was the most important pathogen.

In vitro antimicrobial susceptibility test result

Antimicrobial sensitivity test was done for all of the bacterial isolates in which *S .aureus* showed high resistance to penicillin (75%), polymyxin (80%), bacitracin (82%) and amoxicillin (75%). But it was sensitivity to gentamicin (92%), kanamycin (95%), chloramphenicol (65%), streptomycin (80%), vancomycin (85%).

In the present study *S. intermidius* were found sensitive to all antimicrobial disks. CNS isolates were highly resistant to Penicillin (70%), Bacitracin (90%) and Polymyxin (75%) and highly sensitive to Chlramphenicol (100%) and Vancomycin (81%). The test also indicated that *S. agalactaie* was highly resistance to amoxicillin (50%), Polymyxcin (90%) and highly sensitivity to Gentamicin (100%), Vancomycin (100%), Penicillin (84.6%), Chloramphenicol (95%) and Streptomycin (72%).

S. dysgalctiae was highly sensitive to almost all antimicrobial disk applied except for amoxicillin, for which it was highly resistant. Isolate of *S. uberis* was resistant to Bacitracin (70%), Amoxicillin (65%) and highly sensitive to almost all antibiotic disks, whereas *E.coli* isolates were sensitive to all antimicrobial disks except Penicillin, Amoxicillin, Bactracidin and Polymixin. *Bacillus species* were highly sensitive to almost all antimicrobial disks applied except for Penicillin and Bacitracin.

DISCUSSION

Prevalence of bovine mastitis

The overall prevalence of clinical mastitis accounted 5.9% based on CMT and clinical examination at cow level, which is in consistent with 3.0% prevalence reported by Gizat et al. (2007). The variability in the prevalence of bovine mastitis is due to interaction of several factors mainly of management, environment and factors related to animal and causative organism. The present

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

Observation level	N	Prevalence subclinical mastitis in % (n)		Prevalence of clinical mastitis in % (n)	
		CMT (n)	Culture (n)	Clinical observation (n)	Culture (n)
Heard	46	60.8(28)	50(23)	17.3(8)	17.3(8)
Cow	200	25 (50)	24(48)	3.0(6)	3.0(6)
Quarter	793	12.7 (101)	9.1(91)	1.2(10)	1.2(10)

N: number examined; n: number positive.

Table 2. Risk factors affecting the prevalence of subclinical mastitis at cow and herd level.

Factor	Category	N	Number positive (%)	P value	OR	95% CI of OR
Herd size	1-5	134	39 (29.1)	0.568	0.84	0.47-1.51
	> 5	66	17 (25.8)			
Stage of lactation	Beginning	32	3 (9.4)	-	-	-
	Middle	125	16 (12.8)	0.643	1.41	0.32-6.23
	End	43	37(86.1)	0.000	59.61	11.58-306.80
Parity	1-3	121	12 (9.9)	0.000	11.42	4.89-26.65
	> 3	79	44 (55.7)			
Presence of teat lesion	Yes	5	4 (80.0)	0.032	11.00	1.23-98.69
	No	195	52 (26.7)			
Herd level						
Herd size	1-5	131	94 (71.76)	0.361	1.84	0.50-6.76
	>5	17	14 (82.35)			
Udder washing before milking	Yes	72	49 (68.06)	0.192	0.61	0.30-1.28
	No	76	59 (77.63)			
Milking mastitis cow last	Yes	35	9(25.7)	0.00	0.05	0.02-0.13
	No	113	99(87.6)			
Hand washing before milking	Yes	7	5(71.4)			
Drainage structure	Good	90	61 (67.78)	0.079	2.03	0.92-4.48
	Bad	58	47(81.0)			

the treatment of clinical cases, however, sub clinical form of mastitis was neglected which is responsible for high economic loss (Kerro, and Tareke, 2003). Though dairy farming is mostly a sideline business for the owners of small holder dairy farms in the study areas, they were not well informed about the invisible loss from sub clinical mastitis.

Bacterial isolation and identification

In the present study *S. aureus* was the predominant pathogen (38.9%) compared to all isolates in the area which is comparable with the findings of Workneh et al. (2002) and Barbuddahe (2001) who reported 39% and 38.8% isolates of *S. aureus* respectively. However, the current finding is in contrary to previous 9% research reported by Gizat et al (2007). Similar study conducted in Jamaica and India by Zingeser et al. (1991) and Barbuddahe et al. (2001) indicated lower *S. aurues* isolates which accounted 27% and 23.2% respectively than the current finding. The relative high prevalence of *S. aureus* in this study could be associated with lack of

Table 3. Bacterial isolates from quarters affected by clinical and subclinical mastitis.

Bacterial isolates	Clinical (%)	Subclinical (%)	Total (%)
S. aureus	2(22.2)	30(38.9)	32(37.2)
CNS	2(22.2)	18 (23.4)	20 (23.3)
S. intermidius	-	4(5.2)	4 (4.7)
S. agalactaie	3 (33.3)	5(6.5)	8 (9.3)
S. dysgalactaie	1 (11.1)	1(1.3)	2(2.3)
S. uberis	-	1(1.3)	1 (1.2)
E. fesalis	-	-	-
Bacillus species	1 (11.1)	8(10.4)	9 (10.5)
E. coli	-	6(7.8)	6 (7.0)
Enterobacter species	-	-	-
Klebssela species	-	-	-
Micrococcus	-	1(1.3)	1(1.2)
C. bovis	-	1(1.3)	1(1.2)
A. pyogens	-	2(2.6)	2 (2.3)
Total	9(100)	77 (100)	86(100)

effective udder washing, hand washing before milking, use of separate towel, post milking teat dipping and disinfection routine milking area. Kerro and Tareke (2003) indicated 40.5% *S. aureus* prevalence in southern Ethiopia and 44.4% in Sebeta Miline et al. (2002) which are higher than the present finding.

The result of coagulase negative staphylococcus (CNS) in present study accounted 23.2%, which is lower than 42% (Hussien, 1999) and 46% (Gizat et al., 2004) findings. However, it is higher than 10% prevalence reported (Miline, 2002). CNS is regarded as minor pathogen and normally considered as normal inhabitants of bovine udder (Gentilini et al., 2002).

The prevalence of *S. agalactaiae* in this study is lower than 13.1% reported by Kerro and Tareke (2003). Gizat et al. (2007) indicated a 1.5% prevalence of *S. uberis* which is in consistence with the current findings. Isolates of *S. dysgalactaie* were higher than 0.5 and 5.6% indicated by Gizat et al. (2007) and Kerro (1997), respectively. *E. coli* which was the predominant isolate in the current study might be associated with poor farm cleanness and stable areas. This study also showed that environmental pathogens were isolated in similar proportion. An increased herd size, poor manure disposal and sanitation problem leads to the building up of bacterial population such as coliform and environmental *Streptococcus* in the cows' immediate environment.

In-vitro antibiotics sensitivity test

S. aureus, the major cause of mastitis were found sensitive to gentamicin (92%), chloramphenicol (65%), kanamycin (95%), vancomycin (82%) and highly resistant to bacitracin (100%), polymyxin (80%), penicillin (89%) and amoxicillin (75%). This finding is in close agreement with the findings of Edward et al. (2002) who reported *S. aureus* was highly resistant to bacitracin and amoxicillin at 94 and 74%, respectively. Sanmartin et al. (2007) report *S. aureus* was found resistant to amoxicillin (60%) and penicillin which was in accordance with 68% current findings. Similar study in Argentina indicated that *S. aureus* was found highly susceptible to gentamicin (90%) and chloamphenicol (Gentilini et al., 2002). According to Kang et al. (2007), *S. aueus* and *Streptococcal* species including CNS were highly resistant to penicillin, which is similar to the current study that may be due to the long term use of beta-lactam antibiotics in intra-mammary infusion therapy.

Sanmartin et al. (2007) indicated that CNS strain were resistant to penicillin (56%) and amoxiciline (42%), this result is comparable to the present study in which CNS isolates were resistant to amoxicillin (35%). *S. agalactiea* in this study showed 100, 95, 85, 50 and 100% sensitivity to gentamicin, chloramphenicol, penicillin, kanamycin and vancomycin, respectively; however, they are resistance to amoxicillin (50%) and polymyxin (90%). *S. agalactaie* were 100% sensitive to gentamicin according to Shakuntala (2003), which is in agreement with the current findings. Shakuntala (2003) also indicated 75% sensitivity to chloaphenicol, which is comparable with the present finding.

The study also revealed that *E. coli* isolates was sensitive to chloraphenicol (100%), kanamycine (78%), gentamicin (80%), streptomycin (78%) and vancomycin (100%), whereas resistant to penicillin (65%) and bacitracine (92%). These results are close to the report in India (ICAR) in which *E. coli* was found to be 100%

sensitive to chloramphenicol and 50% to gentamicin (Shakuntala et al., 2003).

Conclusion

The current finding indicated an occurrence of low to moderate prevalence of clinical mastitis and moderate to high prevalence of subclinical mastitis at cow, herd and quarter level. Stage of lactation, parity number and presence of teat lesion were the most important risk factor affecting the prevalence of sub clinical mastitis in cow level and milking mastitic cow at least at herd level. *S. aureus* was the predominant pathogens in clinical and subclinical mastitis. *S. agalactaie* was the most frequently encountered bacteria during clinical mastitis. All the current Isolates were sensitive to gentamicin, kanamycin, chloamphenicol and vancomycin; moderately sensitive to streptomycin and penicillin. However, the isolates were resistant to bacitracin and polymixin which is similar to different researches made on similar isolates. Since mastitis is an economically important disease, hygienic milking practice, use of effective antibiotics, proper extension packages to dairy farm owners and strategic mastitis control programs should be of paramount importance.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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