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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL PROFILE OF *CORYNEBACTERIUM BOVIS* FROM SELECTED DAIRY FARMS IN BISHOFTU, CENTRAL ETHIOPIA

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Abstract

From January to May 2018, a cross-sectional study was undertaken on lactating dairy cows in Bishoftu town to isolate *Corynebacterium bovis*, determine the prevalence and risk factors, and evaluate the effectiveness of several antibiotics in lactating dairy farms. Study animals were selected randomly from selected dairy farms in the area. Collecting milk samples from mastitic cows, cultivating, and then performing an antibiotic sensitivity test were the procedures followed. A total of 384 lactating dairy cows were examined with inspection and California Mastitis Test (CMT), in which 86 of them were found to be CMT positive. Accordingly, prevalence was 3.9% and 18.5% for cows affected by clinical and subclinical mastitis respectively. The prevalence of mastitis showed statistically significant difference between, lactation stage, breed, age and washing ($p > 0.05$). However, there was no statistically significant difference noted in animal husbandry practice ($p > 0.05$). A total of 384 lactating dairy cows were examined with inspection and CMT, in which 86 of them were found to be CMT positive. Out of the 86 mastitis positive samples (sample indicates milk from one cow) sent to microbiology laboratory for microbiological examination, 7 bacterial isolates were identified as *Corynebacterium bovis.* The biochemical and morphological characteristics of 7 (1.8%) *C. bovis* isolated from bovine milk samples and the *C. bovis* reference strains were found to be uniform. Valuable criteria for identification were presence of catalase and oxidase,

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production of acid from glucose and a requirement for enriched basal media. *C. bovis* isolates have revealed a higher sensitivity to the kanamycin and streptomycin (71.4% each). A certain resistance has been noted to oxytetracycline (71.4%) and nalidixic acid (42.8%). Higher number of isolates showed moderate sensitivity or resistance to amoxicillin (51.1%). Regarding to multidrug resistance, the study reflects that only one isolate (14.3%) shows multidrug resistance to four drugs namely kanamycin, amoxicillin, nalidixic acid and oxytetracycline. This study demonstrated that mastitis due to *C. bovis* is rare in lactating dairy farms in Bishoftu. Some of the risk factors for mastitis can be addressed by practical management of dairy cows. Farm owners should selectively use the antibiotics to which the bacteria do not show resistance, such as streptomycin and kanamycin.

Key words: *Corynebacterium bovis*, CMT, dairy farm, mastitis, prevalence

IZOLACIJA, IDENTIFIKACIJA I ANTIMIKROBNI PROFIL *CORYNEBACTERIUM BOVIS* SA ODABRANIH FARMI MLEČNIH KRAVA U BISHOFTU, CENTRALNA ETIOPIJA

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Kratak sadržaj

U periodu od januara do maja 2018. sprovedena je studija preseka na mlečnim kravama u fazi laktacije u gradu Bishoftu. Ciljevi istraživanja bili su izolacija *Corynebacterium bovis*, određivanje prevalence i faktora rizika, kao i procena efikasnosti nekoliko antibiotika na mlečnim farmama tokom perioda laktacije. Ispitivane životinje odabrane su sa farmi u datoj oblasti metodom slučajnog izbora. Ispitivanje je vršeno u skladu sa procedurama koje su obuhvatile prikupljanje uzoraka mleka od krava sa mastitisom, kultivaciju i sprovođenje testa osetljivosti na antibiotike. Ukupno je ispitano 384 mlečne krave u laktaciji, što je podrazumevalo pregled i California Mastitis Test (CMT), gde je ustanovljeno da je 86 životinja bilo CMT-

pozitivno. Shodno tome, utvrdjen je stepen prevalencije od 3,9% kod krava sa kliničkim i 18,5% kod krava sa subkliničkim mastitisom. Utvrđene su statistički značajne razlike u pogledu stope prevalentnosti mastitisa u odnosu na stadijum laktacije, rasu, starost i pranje vimena ($p > 0.05$). Statistički značajne razlike nisu ustanovljene u odnosu na način uzgoja ($p > 0.05$).Od ukupno 86 uzoraka pozitivnih na mastitis (uzorak se odnosi na mleko od jedne krave) poslatih na ispitivanje u mikrobiološku laboratoriju, 7 bakterijskih izolata identifikovano je kao *Corynebacterium bovis.* Ustanovljeno je da se biohemijske i morfološke karakteristike 7 (1,8%) izolata *C. bovis* izolovanih iz uzoraka kravljeg mleka podudaraju sa referentnim sojevima *C. bovis*. Prisustvo katalaze i oksidaze, produkcija kiselina iz glukoze i potreba za obogaćenim hranljivim podlogama primenjeni su kao validni kriterijumi za identifikaciju bakterijskog mikroorganizma. Izolati *C. bovis* pokazali su veću osetljivost na kanamicin i streptomycin (71,4% na oba). Uočen je određeni stepen rezistencije na oksitetraciklin (71,4%) i nalidiksičnu kiselinu (42,8%). Veći broj izolata pokazao je umerenu senzitivnost ili rezistentnos na amoksicilin (51,1%). Što se tiče multiple rezistencije na lekove, studija je pokazala da je samo jedan izolat (14,3%) bio rezistentan na četiri leka – kanamicin, amoksicilin, nalidiksičnu kiselinu i oksitetraciklin. Ovo istraživanje je pokazalo da je mastitis izazvan *C. bovis* retka pojava na farmama mlečnih krava u fazi laktacije u oblasti grada Bishoftu. Neki od faktora rizika za mastitis mogu se kontrolisati kroz adekvatno upravljanje na farmi. Vlasnici farmi bi trebalo selektivno da koriste antibiotike na koje bakterije ne pokazuju rezistenciju, na primer streptomicin i kanamicin.

Ključne reči: *Corynebacterium bovis,* CMT, farma mlečnih krava, mastitis, prevalentnost

INTRODUCTION

Ethiopia has high livestock production potential and is a home for around 61.6 million cattle (55.23% female and 44.77% male), 69.7 million sheep and goats, 11.7 million equines, 48.2 million poultry and 3.8 million camels. However, the country is not benefiting from the livestock sector as its production potential. Along with drought, feed and water shortage, and genetic factors of the animals, animal diseases are the most common constraints to the sector in particular and the country in general. Livestock diseases can cause death of animals, loss of weights, slow down growth, poor fertility performance, decrease in physical power and the likes (CSA, 2020). Bovine mastitis is the sec-

ond most frequent disease next to reproductive disorders and one of the major causes for economy failure in Ethiopia. It affects both the quantity and quality of milk. Mungube (2001) calculated the cost of mastitis in Addis Ababa's urban and peri-urban districts to be 210.8 Ethiopian Birr per cow per lactation. Aside from the financial implications, there is a risk that bacterial contamination of milk from infected cows will make it unfit for human consumption by causing food poisoning or providing a pathway for disease transmission to humans. Brucellosis and tuberculosis can be transmitted to humans in this way (Radostits et al., 2000).

In Ethiopia, mastitis prevalence rate was 85.6% and 81.2% using CMT and somatic cell count (SCC), respectively (Husien et al., 1999). An overall prevalence of 30.2% and 5.5% was obtained for subclinical and clinical mastitis, respectively, in a study conducted in urban and per-urban dairy production system in and around Addis Ababa. In addition, 43 and 75% prevalence rates of bovine mastitis were reported in different parts of Ethiopia (Mekibib et al., 2010).

Mastitis induced via pathogenic microorganisms generally come from two sources: the environment such as *Escherichia coli*, *Enterobacter* and *Klebsiella* acquired by exposure of the teat to contaminated environment, or the animal itself like *Staphylococcus aureus* and *Streptococcus agalactiae*, Coagulase negative *Staphylococcus, Micrococcus* species, *Corynebacterium* species, *Bacillus* species, *Pasteurella* species, *Mycoplasma* etc. (Workineh et al., 2002). *Corynebacterium bovis* is the most frequently isolated *Corynebacterium* spp. from bovine intramammary infections (IMI) (Woodward et al., 1990). *C. bovis* readily colonizes the teat canal of dairy cows and has been used as an indicator of milking hygiene. It is not uncommon for *C. bovis* to be isolated from over 60% of quarter milk samples in herds where post milking teat antisepsis is not used. Indeed, the rate of new *C. bovis* IMI was about 30 times higher than that of *Streptococcus agalactiae* under experimental challenge settings. However, rather than real IMI, this high infection rate was thought to be attributable to teat canal colonization and subsequent contamination of milk samples (Woodward et al., 1990). This high reliance on presumptive identification has limited the ability of most mastitis microbiology laboratories to recognize *Corynebacterium* spp. (Hogan et al., 1999).

In recent years, antimicrobial resistance has been a 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 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. Susceptibility tests of milk samples submitted to state diagnostic laboratories that use the disk-diffusion method have demonstrated remarkable agreement but vary from results of a small survey processed using broth dilution (Constable and Morin, 2003).

Ethiopia holds large potential for dairy development due to its large livestock population the favorable climate for improved high-yielding animal breeds, and the relatively disease free environment for livestock. Given the considerable potential for smallholder income and employment generation from high value dairy products, development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country (Ahmed, et al., 2003). Various authors have indicated that mastitis is a major problem in Ethiopia. However, works on pathogen specific mastitis particularly that of *C. bovis* and their effect on milk production is insufficient. Therefore, the objectives of this study were: To isolate and characterize *C. bovis* and test its antimicrobial susceptibility from dairy cows with mastitis, and to estimate prevalence of mastitis in lactating dairy cows in Bishoftu dairy farms.

MATERIAL AND METHODS

Study Area

The study was conducted in Bishoftu town from January to May 2018. Bishoftu is located at 9°N and 40°E, in Oromia National Regional State about 47 km southeast of the capital city of Ethiopia, Addis Ababa. The altitude is about 1850m above sea level. It experiences a bimodal pattern of rainfall with the main rainy season extending from June to September (of which 84% of rain is expected) and a short rainy season from March to May with an average annual rainfall of 800mm. The mean annual minimum and maximum temperatures are 12.3°C and 27.7°C, respectively, with an overall average of 18.7°C. The highest temperatures recorded in May and the mean relative humidity is 61.3%. Bishoftu is the center of Ada‟a Liben woreda. The Woreda has a total land area of about 1610.56 Km^2 and is divided into three agro-ecological zones: the midland (94%), highland (3%) and lowland (3%) (ADARDO, 2011).

Study Animals

The study populations included milking cows found in Bishoftu, selected from smallholder (< 5 cows), medium sized (5-16 cows) and large sized (>16 cows) private and government-owned dairy farms. The sampling animals were selected randomly from the selected dairy farms in the town.

Study Design

A cross-sectional study was carried out from January 2018 to May 2018 and bacteriological analysis of milk samples from mastitis infected dairy cattle found in Bishoftu town was performed.

Sample size

The total numbers of study animals required for the present study were calculated based on the formula given by Thrusfield (1995). As there was no previous information available in the study area, 50% expected prevalence was taken for sample size determination. Moreover, 5% level of precision and 95% of confidence interval were used to calculate the sample size. Accordingly, a total of 384 animals were considered as sample size during the study period.

Milk Sample Collection

Composite samples of approximately 10 ml of fresh milk were collected from each cow before milking using sterile tight-seal sampling bottles to avoid leakage and contamination. From each farm, one sample per animal was taken by mixing the milk from all quarters. Before beginning with sample collection, loose dirt, bedding, and hair from the udder and teats were brushed with a hand and excessively dirty udder, the teats were washed with lukewarm water thoroughly dried with a towel, and disinfected with 70% ethyl alcohol. Before collecting milk samples from each quarter, the first two streams of milk were discarded, and the milk and udder were examined for evidence of clinical mastitis. Between milking two cows, hands were cleansed in a sanitizing solution, and gloves were used if contagious diseases were anticipated. (Quinne et al., 1999). The sample was taken and stored in an ice box before being transported to the Microbiology Laboratory of the College of Veterinary Medicine and Agriculture for bacterial culture and isolation.

CMT Screening

Abnormal milk, milk clots, gland swelling, and cow disease are the signs of clinical mastitis, while CMT was used to identify subclinical mastitis. CMT was carried out according to the method described by Quinne et al., (1999). Each of the four shallow cups in the CMT paddle received roughly 2 ml of milk sample from each quarter. On each cup, an equal amount of CMT reagent was added, and the mixtures were gently rubbed together in a horizontal plane for no more than 15 seconds. The existence of subclinical mastitis was revealed by the coagulation and viscosity of the mixture.

Bacteriological techniques

Positive milk samples (clinical and sub-clinical) were collected from cows and cultured bacteriologically following standard microbiological procedures (Quinn et al., 1994). For primary isolation, cultivation, and detection of bacterial hemolytic reaction, blood agar (BBL^{R}, Becton Dickinson, USA) was prepared. Bacteria were isolated by streaking one standard loop of milk (0.01ml) over the surface of blood agar supplemented with 5% sheep blood. The inoculated blood agar plates were placed in an incubator at 37°C for 48 h. Identification of Corynebacteria isolated from bovine mammary glands has been largely based on colony morphology, hemolysis, and growth requirements. The presence of small, white and non-hemolytic colonies on 5% sheep blood agar after 48 h of incubation at 37°C indicates *C. bovis.* Furthermore, *C. bovis* tends to grow nicely only in regions of visible milk fats because it requires oleic acid. From subculture blood agar plates, colonies were streaked onto nutrient agar (Oxoid, Hampshire, England) for better colony characterization, biochemical checks and sample preservation. The presumptive Corynebacterium colonies, Gram positive, catalase positive and oxidase positive bacilli that passed via Gram stain, catalase and oxidase checks have been similarly purified by sub culturing onto nutrient agar and the plates were incubated aerobically at 37°C for 24 h.

The differentiation between *Corynebacterium* and other mastitis-causing microorganisms were performed using a tube triple sugar iron (TSI) test and Methyl Red broth. Corynebateria can overcome the buffering potential of the media by producing massive quantities of a stable acid end product from glucose fermentation, hence lowering the PH.

Antimicrobial susceptibility test

The antimicrobial resistance patterns of the isolates were determined according to Kirby-Baur disc diffusion method (Quinne et al., 1999, NCCLS, 2012). Antimicrobial susceptibility tests were performed using a broth micro dilution method (Sensititre, Westlake, Ohio), except that the Mueller-Hinton agar was not supplemented with 1% Tween 80 (Watts and Rossbach, 2000). The antimicrobial susceptibility test panel contained the following antimicrobial agents: kanamycin (30µg), streptomycin (10µg), amoxicillin (10µg), nalidixic acid (30µg), oxytetracline (30µg), and ceftriaxone (30µg) (Thermo Scientific™, Oxoid™) were tested by disc diffusion on Mueller-Hinton agar plates (BBL®, Becton Dickinson, USA).

A suspension with a density equivalent to that of a 0.5 McFarland standard inoculums was prepared in 0.9% saline to achieve a final density of the standard. The suspension was applied onto the surface of the Mueller-Hinton agar plates with a swab, and antibiotic disks were applied onto the surface of the inoculated Mueller-Hinton agar plates using aseptic technique (Quinne et al., 1994). The results were recorded after 24 h of incubation at 37°C and interpreted according to the guideline (NCCLS, 2012).

Data Management and Analysis

The data obtained from this study were compiled, entered to Microsoft Excel work sheet and analyzed with Statistical Package for Social Science (SPSS) 20. Descriptive statistics such as percentage, frequency and cross tabulation distribution were used to describe the nature and characteristics of generated data on the rate of bacterial isolation and resistant patterns of the bacterial isolates. A confidence level of 95% was used to interpret statistical associations. Categorical variables were compared by using chi-square tests. *P* - Value was calculated and $p > 0.05$ was taken as statistically significant.

RESULTS

A total of 384 lactating dairy cows were examined with inspection and CMT for detection of clinical and subclinical mastitis, respectively. Of the total lactating cows examined, overall mastitis prevalence in the area was 22.4% (68/384). The results showed that the prevalence rates of clinical and subclinical mastitis were 3.9% and 18.5%, respectively (Table 1).

Table 1. The overall prevalence of bovine mastitis

Mastitis condition	No. cows Examined	No. of positive (%)
Clinical mastitis	384	15 (3.9)
Subclinical	384	71 (18.5)

The result showed that the effect of lactation stage was statistically significant ($p > 0.05$) for the prevalence of bovine mastitis, and the infection rate was high in animals in early (49.1%) and late (68.4%) lactation stage as compared to the mid lactation stage (3.1%). Animals managed in semi-intensive husbandry practice showed high rate of infection (27.8%) as compared to those managed intensively (21.8%). The infection rate was lower in crossbred dairy cows (7.1%) than in those of Holstein Friesian (75%) and local breeds (11.8%). The other variable in the study was the age, and the study revealed the following values for dairy cows < 3 years (1.2%), 4-8 years (11.4%) and those > 8 years (81.9%), thus, as the age increases the incidence of mastitis also increases. In the present study, from selected potential risk factors the breed ($p > 0.05$), stage of lactation ($p > 0.05$), and age ($p > 0.05$) had statistically significant effect, but animal husbandry practice had no significant effects on the prevalence of mastitis ($p > 0.05$) (Table 2).

Table 2: Prevalence of bovine mastitis in relation to lactation stage, breed, husbandry and age

Risk factors		No. of cows examined	No. of positive (%)	X^2	*P* - value
Lactation Stage	Early	53	26 (49.1)	167.8	0.00
	Mid	255	8 (3.1)		
	Late	76	52 (68.4)		
Husbandry	Intensive	348	76 (21.8)	0.662	0.406
	Semi-intensive	36	10 (27.8)		
Breed	Holstein Friesian	84	63 (75)	171.56	0.00
	Cross-bred	266	19 (7.1)		
	Local	34	4 (11.8)		
Age	<3 years	83	1 (1.2)	184.41	0.00
	4-8 years	229	26 (11.4)		
	>8 years	72	59 (81.9)		

In the majority of mastitis, bacteriological techniques are used for phenotypic characterization to presumptively identify *C. bovis*. Basically, the organisms that exhibit a small white non-hemolytic colony type after 48 h of incubation in the area where butterfat was deposited on agar surface were presumptively considered to be *C. bovis*.

Out of 86 CMT positive samples, 57 (66.3%) organisms were identified presumptively as coryneform bacteria and had similar biochemical reactions and were consistent with the *C. bovis* reference strain. All or almost all were small, circular with regular edges, white to cream in color, and non-hemolytic after 48 h incubation on blood agar enriched with 5% sheep blood. Colonies usually appear on the first strike, which is due to the lipophilic nature of the bacterium.

All strains were Gram positive, catalase positive and oxidase positive. The remaining 29 strains were identified as coryneforms based on Triple Iron Sugar (TSI) test. The study showed that coryneform bacteria ferment glucose and cause acid production, but no acid production was detected using carbohydrates lactose and sucrose as substrates (Table 3). The isolates identified as coryneform bacteria were further identified based on Methyl Red and Voges-Proskauer (MR-VP) test. Gas production was detected in 2 isolates.

Table 3: Summary of the metabolic and physiological characteristics of presumptive *C. bovis*

Test or Substrate	***C. bovis* isolates**	**No. of isolates**
Growth on blood agar with 5% sheep blood	+	
Morphology on blood agar	Small white, non-hemolytic colony	54
Gram stain	Gram positive, rod shaped	45
Catalase test	+	40
Oxidase test	+	36
Acid production from		
Glucose	+	29
Lactose	-	
Sucrose	-	
Methyl Red	-	7

Key: (+) = positive, (-) = negative

Information on the susceptibility of coryneform bacteria to antimicrobial agents is scarce. The study of the frequency of susceptibility of *C. bovis* (n = 7) to antibiotics revealed higher sensitivity to kanamycin and streptomycin (71.4% each). A certain resistance has been noted to oxytetracycline (71.4%) and nalidixic acid (42.8%). Higher number of isolates showed moderate sensitivity or resistance to amoxicillin (51.1%). Regarding to multidrug resistance, the study reflects that only one isolate (14.3%) shows multidrug resistance pattern. Susceptibility and resistance patterns of each bacterial isolates are shown in Table 4.

Table 4: Frequency of susceptibility of *Corynebacterium bovis* (n = 7)

Bacterial strains		***Corynebacterium bovis* (n=7)**	
Antibiotic agent	S %	IM %	R %
Kanamycin	71.4	14.3	14.3
Streptomycin	71.4	0	28.6
Amoxicillin	14.3	51.1	28.6
Nalidixic acid	28.6	28.6	42.8
Oxytetracycline	14.3	14.3	71.4
Ceftriaxone	28.6	42.8	28.6

Key: S=Susceptible, I= Intermediate, R=Resistant

From the total *C. bovis*, 14.3% were resistant to one drug, 57.14% to two drugs and 14.3% were to more than two drugs.

DISCUSSION

This study showed the overall prevalence of mastitis associated with *Corynebacterium bovis* in lactating dairy farms to be 1.8%, which is lower than most of the previous reports in Ethiopia and is in agreement with the bovine mastitis reported by Dabash et al. (2014) with the prevalence of bovine mastitis of 2% in North Showa of Ethiopia. This finding is also comparable with the findings of Belayneh et al., (2014) in East Showa zone, Akaki district, Ethiopia (1.2%); Moges et al., (2011) in and around Gonder (2.4%) and Oalekish et al. (2013) in northern Jordan (3.9%). This finding slightly differed from earlier investigations. In other regions in Ethiopia, Lidet et al. (2013) reported 0.52% isolation rate. The high isolation rate in this study could be associated with lowered resistance of the cow due to teat injury.

Clinical mastitis rate was low in all breeds as compared to subclinical mastitis. The prevalence of clinical mastitis in the present study (3.9%) was comparable to the reports from different dairy farms: 6.3% in Bahir Dar (Gizat et al., 2007), and 7.9% in commercial farms in Ethiopia (Abaineh and Sintayehu, 2001). However, the present finding is by far lower than the reports of Kerro and Tarekegn (2003) in local, Friesian and Jersey cows in Sothern Ethiopia (12.1%), and 14.2% in lactating cows in smallholder farms in Tanzania (Kivaria et al., 2004). Mastitis is a complex disease and the difference in results could be due to variations in herd size, management practices, proportion of exotic gene inheritance, and agro-climates. Other risk factors might also have contributed to the observed differences in prevalence rates of mastitis among the findings of various authors.

Significant differences between the high-grade Holstein-Friesian, Holstein indigenous zebu crossbred and local zebu might be associated with their high milk yield. Radostits et al. (2007) stated that high-yielding cows are more susceptible to mastitis than the low-yielding ones.

Significant effect of stage of lactation on the prevalence of mastitis was confirmed in this study, being 49.15%, 3.1% and 68.4% in early, mid and late lactation, respectively, also reported by Nesru (1999), Mungube et al. (2005) and Kerro and Tarekegn (2003) in Ethiopia. The former two authors reported high prevalence of subclinical mastitis in cows at early and late stage of lactation as it is the case in this finding, while the last two reported higher prevalence at early stage of lactation. The variations in the effect of lactation stages between different studies could be related to the disparities in age, parity and breed of the sampled animals.

The present study was also undertaken to determine the resistance pattern of bovine mastitis due to *C. bovis* to commonly used antimicrobials in the study area and to provide information to concerned animal health professionals. The selection of the types of antimicrobial agents was based on clinical considerations including frequent use of the drug in the study area and availability. Oxytetracycline was commonly used antimicrobial for the treatment of mastitis in the study area.

The poor inhibitory effect of oxytetracycline against *C. bovis* strains identified in this study is in agreement with the data reported by Oalekish et al. (2013). The latter reported sensitivity to oxytetracycline in 12% of *C. bovis* isolates from subclinical mastitis in northern Jordan. In our study, *C. bovis* isolates showed most susceptibility to kanamycin and streptomycin (71.4% each). Nibret et al. (2011) obtained comparable results, where kanamycin was effective against *C. bovis* (100%). In the present study, unlike Oalekish et al. (2013), streptomycin was effective against *C. bovis* (71.4%). Out of the seven isolates

investigated in the present study, only one isolate (14.3%) showed multidrug resistance to kanamycin, amoxicillin, nalidixic acid and oxytetracycline.

In general, streptomycin and kanamycin showed very good efficacy, nalidixic acid and ceftriaxone showed moderate efficacy, whereas oxytetracycline and amoxicillin showed poor efficacy in almost all isolates.

CONCLUSION AND RECOMMENDATIONS

Bovine mastitis is an inflammatory response of the mammary gland. It has a major impact on animal production, animal welfare and milk quality. Mastitis is one of the biggest problems for dairy industry because of high morbidity and significant economic losses. Even though, the prevalence of *C. bovis* is lower than that of other common pathogens, it causes mostly subclinical mastitis and has substantial impact on the dairy industry. Milk from cows with subclinical mastitis accidentally mixed into bulk milk enters the food chain, and poses a threat to human health. The low prevalence of C. *bovis* did not restrict the organism to show resistance to antibiotics such as oxytetracycline, nalidixic acid and others, emphasize the need for serious and immediate attention towards consumers of raw milk. On the other hand, some antibiotics such as kanamycin and streptomycin were found to be effective against *C. bovis.* The appropriate and principled use of those antibiotics might help in the control and limitation of resistance against those strains. Based on the above finding and closing remarks, the following recommendations are forwarded:

- Future work need to be done in the field of isolation, characterization and prevalence of coryneform bacteria and consequently, the responsible important strains could be identified.
- Antimicrobial susceptibility test should be conducted at regular intervals to understand the development of resistance against the commonly used antibiotics.
- Proper hygienic and improved management practices should be introduced at farm level.

Author's Contribution:

MY made contributions to conception and design of the study, involved in data collection, data curation, biochemical test and culturing the bacteria, data analysis and drafting the manuscript. JT revised the manuscript critically and together with MY prepared the final draft of the manuscript. Both authors read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interests.

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