

Full Length Research Paper

Study on bovine mastitis with isolation of bacterial and fungal causal agents and assessing antimicrobial resistance patterns of isolated *Staphylococcus* species in and around Sebeta town, Ethiopia

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A cross-sectional study was conducted from December 2016 to May 2017 in and around Sebeta town with the aim of assessing the prevalence of mastitis, isolation of aerobic bacterial and fungal causal agents and assessing antimicrobial resistance pattern of isolated *Staphylococcus* species in dairy cows. From a total of 383 dairy cows, 220 (57.4%) were found to be positive for mastitis of which 10.4% were affected by clinical mastitis and 47% by subclinical mastitis. Mastitis was more likely to occur in cows above 8 years of age (OR = 16.9, 95% CI = 7.8 - 36.00) and in those cows washed by hand before milking (OR = 7.8, 95% CI = 4.2-14.6) as compared to those subjected to udder washing and drying using towels. *Staphylococcus aureus* was the most frequently isolated bacterial species (25%) followed by *Streptococcus agalactiae* (12.3%) and coagulase negative *Staphylococcus* species (10.5%). *Yarrowia lipolytica* (10.9%) and *Candida etchellsii* (7.3%) were the major yeast species isolated, while *Aspergillus* (6.8%), *Mucor* (5.9%), *Penicillium* (3.6%) and *Fusarium* (3.6%) were the major filamentous fungi species identified from the cultured milk samples. The results of antimicrobial susceptibility testing revealed that the isolated *Staphylococcus* species were highly resistant to penicillin G (93.1%) and oxytetracycline (79.3%) but were susceptible to vancomycin (100%), sulphamethoxazole/trimethoprim (96.6%), ampicillin (89.7%) and erythromycin (86.2%). It could be concluded that bovine mastitis is a major challenge to the dairy producers in and around Sebeta towns. Appropriate control and preventative measures must be instituted and dairy farmers and workers must be trained on proper milking and hygiene practices in order to reduce the prevalence of mastitis in this region. The penicillin resistant *S. aureus* could be a source of serious infection in humans as well and hence comprehensive studies including molecular characteristics of drug resistance gene of *S. aureus* especially of methicillin-resistant should be conducted as farm animals, primarily dairy cattle might serves as a reservoir of infection for humans. In 2% of the cases, only fungal species were identified as causes of mastitis, hence further investigation regarding their pathogenicity and contribution to bovine mastitis is needed.

Key words: Antimicrobial resistance, bovine mastitis, bacteria, Ethiopia, fungus, prevalence, Sebeta.

INTRODUCTION

Ethiopia has the largest cattle population in Africa with an estimated population of 57.83 million (CSA, 2016).

Development of the dairy sector in the country can contribute significantly to poverty alleviation and

nutrition (Shapiro et al., 2015). However, currently milk production do not satisfy the countries milk requirement due to multitude of factors such as mastitis and other diseases that lead to significant loss in production (Biffa et al., 2005).

Mastitis occurs worldwide among dairy animals and it has been described to have an extreme economic impact (Al-majali et al., 2008). According to Bhikane and Kawitkar (2000), dairy cattle mastitis contributes up to 70% of reduced milk production, 9% of discarded milk after treatment, 7% of the cost of veterinary services and 14% of premature culling. The disease can be classified as clinical or subclinical (Eriskine, 2001).

The disease is caused by a multitude of etiological agents that includes bacteria, virus, fungi and algae (Wellenberg et al., 2002; Kivaria et al., 2004). The most common bacterial pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, other *Streptococcus* species and coliforms (Sumathi et al., 2008). Other organisms may also include *Arcanobacterium pyogenes*, *Pseudomonas aeruginosa*, *Nocardia asteroides*, *Clostridium perfringens*, *Mycobacterium*, *Mycoplasma*, *Pasteurella* and *Prototheca* species and yeasts (Radostits et al., 2007). Fungal infections account for up to 2 to 13% of all cases of mastitis in cows (Krukowski et al., 2006). Usually mycotic mastitis is unnoticed by clinician in the first attempt of treatment and administration of antibiotics may aggravate fungal mastitis as some of the antibiotics like penicillin and tetracycline act as a source of nitrogen for various species of fungi (Tarfaroosh and Purohit, 2008).

In Ethiopia, available information indicates that bovine mastitis is a serious challenge in dairy farms. Based on one meta-analysis report that evaluated 39 different studies, the overall prevalence of bovine mastitis was 47.0% (95% confidence interval [CI] = 42.0-52.0) of which 8.3% (95% CI = 6.5-10.3) was clinical mastitis and 37% (95% CI = 32.9-40.7) was subclinical mastitis (Getaneh and Gebremedhin, 2017).

Prevalence of bovine mastitis and predisposing factors were assessed 12 years ago in Sebeta towns (Sori et al., 2005). Since then, many dairy farms have been introduced into the area. Moreover, there are many complaints on poor response to treatment of mastitis using common antimicrobial drugs. Furthermore, to the author's knowledge, there are no published literatures on major fungal pathogens isolated from bovine mastitis in Ethiopia. Based on the aforementioned facts, this study was conducted to assess risk factors, causative agents

and to determine antimicrobial resistance patterns of isolated *Staphylococcus* species isolated from bovine mastitis in and around Sebeta town.

MATERIALS AND METHODS

Study area and population

Sebeta town is located around 25 km from Addis Ababa city at 8°55'N latitude and 38°37'E longitude and 2,356 m above sea level. The climate is warm with the average annual temperature of 17.4°C and 1073 mm averages annual rainfall.

The study population comprises of lactating dairy cows that are managed under extensive, semi intensive and intensive farming systems. A total of 383 lactating dairy cows in and around Sebeta town were examined to determine the overall prevalence of mastitis in the area.

Study design and sample size determination

A cross-sectional study was conducted from December 2016 to May 2017 and study cows were randomly selected from extensive, semi intensive and intensive dairy farms in the area. The sample size was determined based on the formula given by Thrufield (2007) considering 5% absolute precision, 95% confidence interval and 52.78% expected prevalence from previous studies in the area (Sori et al., 2005). Therefore, the calculated sample size was 383.

Study methodology

Physical examination of the udder and milk

The udders were first examined visually and then palpated to detect any possible fibrosis, inflammatory swelling and atrophy of the tissue. The size and consistency of the mammary quarter was inspected for the presence of any abnormalities such as disproportional symmetry, swelling, firmness and blindness of the teat canal. In addition, two streaks of milk from each quarter in a strip cup were inspected visually for the presence of any flakes, clots, pus, watery appearance, blood and color change (Radostits et al., 2007).

California mastitis test (CMT) screening

After physical examination, milk samples were tested by California mastitis test (CMT Kit Lot number 67467, ImmuCell, USA). Briefly, a squirt of milk from each quarter of the udder was placed in each of four shallow cups in the CMT paddle and an equal amount of the reagent was added. A gentle circular motion was applied in a horizontal plane. Positive samples show gel formation within a few seconds. The California mastitis test was conducted to diagnose the presence of subclinical mastitis and based on the thickness of the gel formed by CMT reagent and milk mixture 1:1 test results

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were scored as 0 (negative), 1 (weak positive), 2 (distinct positive) and 3 (strong positive). Milk samples with test result of CMT 1 to 3, were classified as evidence of subclinical mastitis (Radostits et al., 2007). If at least one quarter was positive by the CMT then the cow was considered positive for mastitis.

Data and sample collection

A semi-structured questionnaire was used to collect data on risk factors which include age, parity and hygiene of udder, farm owners and milker's. The age of the animals was determined from birth records and asked from owner and categorized as young adult (3 to 5 year), adult (6 to 8 year) and old (8 years and above); parity as few (1 to 2 parities), moderate (3 to 4 parities), and many (4 and above) (Umer et al., 2015). Udder hygiene was evaluated by observing the presence of any cow dung stain or spot on the udder and hind legs and through asking milker's. The practice for keeping the hygiene of udder was divided into four categories; those that do not practice udder washing at all wash by hand without drying, wash using towel without drying, and wash and dry by using towels.

Milk samples were collected following the standard procedures by the national mastitis council (NMC, 2004). After a quarter had been washed with tap water and dried (in cases when there was a considerable amount of dirt), the teat end was swabbed with cotton soaked in 70% ethyl alcohol. Approximately 10 ml of milk sample was then collected aseptically from clinically and sub clinically (CMT positive) mastitic cows into sterile universal bottles after discarding the first milking streams (squirt). The bottles were labeled with permanent marker and transported on packed ice box to National Animal Health and Diagnostic Investigation Center (NAHDIC), where they were immediately cultured or stored at 4°C until processed for bacterial and fungal isolation.

Laboratory work

Bacterial identification

Collected milk samples were inoculated on sheep blood agar (OXOID) and MacConkey agar (OXOID) and incubated aerobically at 37°C and cultured plates were examined after 24 to 48 h of incubation for any visible growth. The colonies were identified using primary and secondary biochemical tests at least to determine the genus of the suspected isolate. Then, OMNILOG/BIOLOG (fully automated coated microplate based bacterial identification system) using GEN III micro plate (Lot number 3003241, BIOLOG, USA) with protocol A method was used to further confirm the species of suspected colonies. A single colony grown in Biolog Universal growth (BUG) agar medium was selected and emulsified into 'inoculating fluid A' (IF A). According to the manufacturer's instructions, cell density of the bacterial inoculum was measured and adjusted for a specified transmittance (90 to 98%) using a turbidimeter. For each isolate, 100 µl of the bacterial suspension was inoculated into each of the 96 well coated micro plates, using 8 channel pipette and incubated aerobically at 33°C for 22 h. The Omni Log identification system automatically reads each microplate and provides species/sub-species identification (ID), and then the results were printed out (OMNILOG, 2010).

Fungal identification

Milk samples were also simultaneously inoculated onto sabouraud

dextrose agar (SDA) and cultured at 26°C for up to 4 weeks (if no visible fungal growth was observed within this period, no growth was recorded). Isolates were examined macroscopically and identified based on colony shape, size, color and growing pattern. For filamentous fungi, slides were prepared from each colony using scotch tape method where transparent scotch tape was lightly pressed to colony and then the tape was fixed to slide that had a drop of lactophenol cotton blue stain. The slides were observed under microscope in X10 and X40 magnification power and were identified at genus level using fungal identification key (Quinn et al., 2002). Yeast colonies were examined microscopically using Gram staining and species level identification was conducted by OMNILOG identification system using yeast microplates.

Antimicrobial susceptibility test

Three to five well isolated colonies of isolated *Staphylococcus* species were transferred to 5 ml nutrient broth and incubated at 35°C for 4 h. The turbidity of the broth culture was adjusted to obtain turbidity optically comparable to 0.5 McFarland standard solution. After adjusting the turbidity, sterile cotton swab was dipped into the suspension and then Mueller Hinton agar plate was inoculated by rotating 60°. Antimicrobials disc were applied on the media using disc dispenser and then incubated for 24 h. Measurement of zone of inhibition was done by using digital caliper. Six antimicrobials were used: Ampicillin, erythromycin, penicillin G, oxytetracycline, vancomycin, sulphamethoxazole/trimethoprim (OXOID discs) (CLSI, 2010).

Data analysis

Statistical analysis was performed using 'STATA, version 12. Mastitis prevalence was calculated by dividing the number of CMT positive cows by the total number of cows tested. Multivariate logistic regression was used to see the association of the potential risk factors with occurrence of mastitis. The strength of the association was measured using odds ratio (OR). Factors with odds ratio greater than one were considered as risk factors and those with odds ratio less than one were protective factor. In all the analysis, P- value lower than 0.05 was considered as significant.

RESULTS

Prevalence of clinical and subclinical mastitis

From a total of 383 dairy cows examined for mastitis, 220 (57.4%) of them were found positive. The details of the types of mastitis and quarter level mastitis were indicated in Table 1. From the total of 1532 quarters examined, 60 (3.9%) were blind (inactive quarters) and 619 (42.1%) were affected by mastitis (Table 1).

The association of risk factors with mastitis

The prevalence of mastitis was higher in older cows than young and adults and the difference was statistically

Table 1. Prevalence of clinical and subclinical mastitis.

| Form of mastitis | Positive cows (%) | Quarters affected (%) |
|------------------|------------------------|-------------------------|
| Clinical | 40/383 (10.4%) | 73/1472 (4.9%) |
| Subclinical | 180/383 (47%) | 546/1472 (37.1%) |
| Total | 220/383 (57.4%) | 619/1472 (42.1%) |

Table 2. Multivariable logistic regression analysis of the association of different potential risk factors associated with mastitis.

| Risk factor | Total examined | No. of positive (%) | Adjusted OR and 95% CI | P-value |
|----------------------------------|----------------|---------------------|------------------------|---------|
| Age | | | | |
| 2-5 | 132 | 37(28.0%) | 1 | |
| 6-8 | 167 | 110(65.9%) | 5.0(2.9 -8.6) | 0.000 |
| >8 | 84 | 73(86.9%) | 16.9(7.8-36.00) | 0.000 |
| Parity | | | | |
| 1-2 | 237 | 102(43.0%) | | |
| 3-4 | 118 | 91(77.1%) | 1.9(.96-3.91) | 0.066 |
| >4 | 28 | 27(96.4%) | 8.3(.92-74.88) | 0.060 |
| Udder preparation hygiene | | | | |
| No washing | 26 | 12(46.2%) | 1 | |
| Washing by hand | 117 | 93(79.5%) | 7.8(4.2-14.6) | 0.009 |
| Washing by cloth | 116 | 80(69.0%) | 5.1(2.8-9.4) | 0.062 |
| Wash and dry | 124 | 35(28.2%) | .4(.12-1.12) | 0.079 |

OR=Odds ratio; CI=Confidence interval.

significant ($P < 0.05$). The disease was more likely to occur in cows above 8 years of age in comparison to younger animals (OR = 16.9, 95% CI = 7.8- 36.00). Similarly, the prevalence was statistically higher ($P < 0.05$) in cows which their udders were washed by hand only before milking (OR = 7.8, 95% CI = 4.2-14.6) as compared to those cows which their udders were washed and dried using towels. The details of factors considered and association with mastitis are summarized in Table 2.

Bacteria and fungi agents isolated from mastitic milk

From 220 milk samples cultured for bacterial and fungal species identification, 41% were positive for bacterial isolates and 2% for fungal species. Mixed bacterial and fungal isolates were observed in 56% of the samples (Figure 1).

The predominant bacterial isolates were *Staphylococcus aureus* with isolation rate of 25% followed by *Streptococcus agalactiae* (12.3%) and coagulase negative *Staphylococcus species* (10.5%). *Yarrowia lipolytica* (10.9%) and *Candida etchellsii* (7.3%) were the

major yeast species observed while *Aspergillus* (6.8%), *Mucor* (5.9%), *Penicillium* (3.6%) and *Fusarium* (3.6%) were filamentous fungi species identified from the cultured milk samples (Table 3).

In vitro antimicrobial susceptibility testing

Antimicrobial susceptibility test was carried on 29 randomly selected *S. species* isolates. *Staphylococcus species* were found to be resistant to penicillin G (93.1%) and oxytetracycline (79.3%) but were highly susceptible to vancomycin (100%), sulphamethoxazole/Trimethoprim (96.6%), ampicillin (89.7%) and erythromycin (86.2%) (Tables 4 and 5).

DISCUSSION

The overall prevalence of mastitis observed in the present study (57.4%) was higher than the reported prevalence 12 years ago (52.78%) from the same study area (Sori et al., 2005). Some eight years ago, Mekibib et

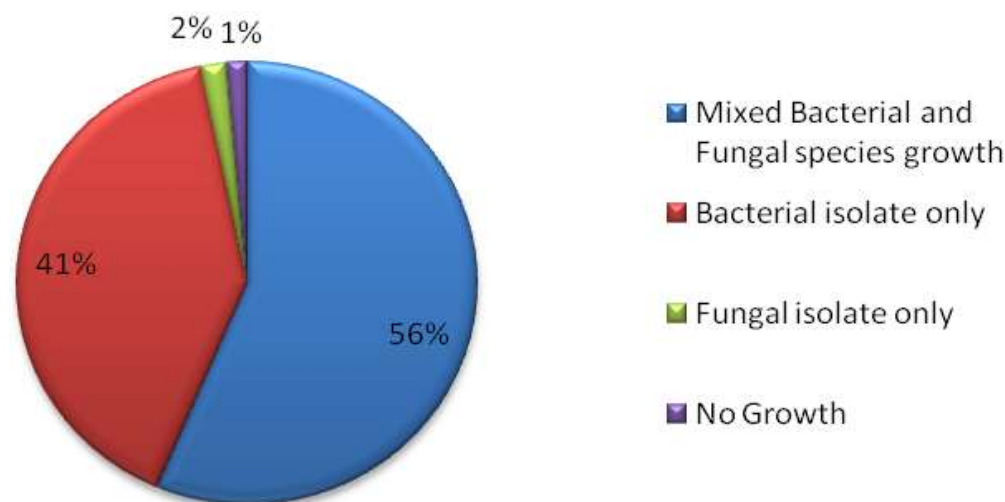


Figure 1. Culture result of milk samples collected from mastitic cows.

al. (2009) reported 71.1% prevalence of bovine mastitis from Holeta, a town which was located close to the present study site. This indicated that bovine mastitis remains a serious problem to the dairy producers in and around these neighborhood towns which costs the farmers from losses associated with reduced production, increased replacement cows, drug costs, veterinary fees and labour costs. It might be due to lack of coordinated actions on prevention and control of bovine mastitis. The numbers of dairy farms has increased in the study areas as compared to previous years. However, most of these farms have poor housing facilities and this might contribute to the contamination and exposure of teats to environmental pathogens and could be reason for increased prevalence of bovine mastitis.

Region to region variations on prevalence of both clinical and subclinical mastitis were wide in Ethiopia. Lakew *et al.* (2009) reported 64.6% overall prevalence from Assela, a town which has similar agro-ecology with the present study site. Mungube *et al.* (2004) and Delelesse (2010) reported 46.6 and 44.1% overall prevalence while Tolosa *et al.* (2009) reported 9.5%, Bedada and Hiko (2011) 12.1%, Sori *et al.* (2005) 16.11% and Workineh *et al.* (2002) 25.1% subclinical mastitis from different localities in Ethiopia. This similarities and differences might be due to complex nature of the disease involving interactions of various factors such as management and husbandry, environmental conditions, animal risk factors, and causative agents (Radostits *et al.*, 2007). The variation in the prevalence of mastitis might also be due to management differences like hygienic condition during milking process practiced by each farm, or individual

cow's defense mechanism (Suriyasathaporn *et al.*, 2000).

The higher prevalence of subclinical mastitis than that of clinical mastitis in the present as well previous studies could be attributed to the little attention given to subclinical mastitis as subclinical mastitis is not clinically visible while treating clinical cases. Moreover, dairy farmers might not be well informed about the silent nature of subclinical mastitis (Karimuribo *et al.*, 2006; Almaw *et al.*, 2008). Likewise, the predominance of subclinical mastitis and its serious economic relevance compared to clinical mastitis was underscored elsewhere out of Ethiopia (Kaliwal and Kurjogi, 2011; Awale *et al.*, 2012; Shittu *et al.*, 2012; Elbably *et al.*, 2013; Katsande *et al.*, 2013).

Quarter level prevalence of mastitis (42.14%) was lower than finding of Kifle and Tolosa (2008) who reported prevalence rate of 63.1%, but higher than the report made by Zelalem (2001) in Ethiopia. The teat canal is the first barrier against invading pathogens, and the efficiency of teat defense mechanisms depends on the integrity of teat tissue; its impairment leads to an increase in the risk of intra-mammary infection. The observed high number of inactive quarter 60(3.9%) may be an indication of a serious mastitis problem on the respective farms and the absence of culling chronically infected cows that can serve as a means to prevent and control the disease within a farm.

In the present study the prevalence of mastitis was higher in old adult cows than young adults. This might be due to older cows have largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agent in the cow's udder (Radostits *et al.*, 2007). Cows with many parities were also at greater risk

Table 3. Bacterial and fungal species identified from milk of cows with clinical and subclinical mastitis.

| Identified bacteria | | Identified yeasts | | Identified filamentous fungi | |
|-----------------------------------|------------|----------------------------------|------------|--------------------------------|------------|
| Species | Number (%) | Species | Number (%) | Species | Number (%) |
| <i>Staphylococcus aureus</i> | 55 (25%) | <i>Candida etchellsii</i> | 16 (7.3%) | <i>Aspergillus species</i> | 15 (6.8%) |
| <i>Staphylococcus intermedius</i> | 23 (10.5%) | <i>Candida edax</i> | 8 (3.6%) | <i>Mucor species</i> | 13 (5.9%) |
| <i>Staphylococcus epidermidis</i> | 12 (5.5%) | <i>Candida heamulonii</i> | 2 (0.9%) | <i>Penicillium species</i> | 8 (3.6%) |
| <i>Staphylococcus hyicus</i> | 11 (5%) | <i>Yarrowia lipolytica</i> | 24 (10.9%) | <i>Fusarium species</i> | 8 (3.6%) |
| CNS | 23 (10.5%) | <i>Rhodotorula graminis</i> | 5 (2.3%) | No growth | 176 (80%) |
| <i>Streptococcus agalactiae</i> | 27 (12.3%) | <i>Rhodotorula glutinis</i> | 2 (0.9%) | Total number of samples | 220 |
| <i>Streptococcus dysgalactiae</i> | 12 (5.5%) | <i>Rhodospiridium diobovatum</i> | 5 (2.3%) | | |
| <i>Streptococcus uberis</i> | 3 (1.4%) | <i>Galactomyces geotrichum</i> | 8 (3.6%) | | |
| <i>Bacillus species</i> | 11 (5%) | <i>Geotrichum terrestre</i> | 3 (1.4%) | | |
| <i>Micrococcus species</i> | 6(2.7%) | <i>Trichosporon species</i> | 7 (3.2%) | | |
| <i>Pseudomonas species</i> | 6(2.7%) | <i>Saccharomyces species</i> | 4 (1.8%) | | |
| <i>Corynebacterium species</i> | 6(2.7%) | No growth | 136 (61.8) | | |
| <i>E.coli</i> | 11(5%) | Total number of samples | 220 | | |
| <i>Klebsiella species</i> | 3(1.4%) | | | | |
| <i>Pasteurella species</i> | 4(1.8%) | | | | |
| No growth | 7 (3.2%) | | | | |
| Total number of samples | 220 | | | | |

CNS = Coagulase negative *Staphylococcus species* other than *S. epidermidis*.

than moderate and few parities which might be due to physical alterations of udder, and is in line with findings reported by other authors (Carlen et al., 2004; Zwald et al., 2004; Abdel-Rady and Sayed, 2009; Belayneh et al., 2013; Katsande et al., 2013, Abrahmsen et al., 2014; Mureithi and Njuguna, 2016).

Cows in farms with poor milking hygiene were severely affected than those with good milking hygiene practices. Similar findings were reported by Sori et al. (2005); Lakew et al. (2009) and Moges et al. (2011). The reason most probably

might be due to cross contamination from infected teat to others, or from infected to non infected cows during milking. The milkers' hand and washing towels might also facilitate pathogens spread. It was also documented that udder preparation both before and after milking influence the prevalence of mastitis.

In this study, the dominant bacterial pathogens isolated from milk samples were *Staphylococcus species* (25%) however; this was lower than that of the 42.1% reported by Abera et al. (2010). Similarly, *S. aureus* was isolated as main

etiological agent of mastitis in cattle in many African and Asian countries (FAO, 2014). *S. aureus* is considered as typical contagious pathogen causing bovine mastitis. Accordingly, the wide spread *S. aureus* mastitis might be cows positive in herd which act as primary reservoir and infected others especially during milking. Radostits et al. (2007) asserted that *S. aureus* is well adapted to survive in the udder and usually establishes a mild sub clinical infection of long duration from which it shed in milk facilitating transmission to healthy animals mainly during

Table 4. Summary of antimicrobial susceptibility test result for *Staphylococcus species* (number of isolates =29).

| Antimicrobial disc | Number of fully susceptible isolates (%) | Number intermediately susceptible (%) | Number of resistant isolates (%) |
|--|--|---------------------------------------|----------------------------------|
| Vancomycin (30 µg) | 29 (100%) | | |
| Ampicillin (10 µg) | 26 (89.7%) | 1 (3.4%) | 2 (6.9%) |
| Erythromycin (15 µg) | 25 (86.2%) | 4 (13.8%) | - |
| Penicillin G (10 Unit) | 1 (3.4%) | 1 (3.4%) | 27 (93.1%) |
| Oxytetracycline (30 µg) | 6 (20.7) | - | 23 (79.3%) |
| Sulphamethoxazole/Trimethoprim (25 µg) | 28 (96.6%) | - | 1 (3.4%) |

Table 5. Penicillin and oxytetracycline resistance patterns of *Staphylococcus species*.

| Isolated <i>Staphylococcus species</i> | Number of tested isolates | Penicillin G | | | Oxytetracycline | |
|--|---------------------------|--------------|---|----|-----------------|----|
| | | S | I | R | S | R |
| <i>Staphylococcus aureus</i> | 13 | - | - | 13 | - | 13 |
| <i>Staphylococcus scuri</i> | 3 | - | 1 | 2 | 3 | 0 |
| <i>Staphylococcus lentus</i> | 2 | 1 | - | 1 | 1 | 1 |
| <i>Staphylococcus xylosus</i> | 4 | - | - | 4 | 2 | 2 |
| <i>Staphylococcus intermedius</i> | 4 | - | - | 4 | - | 4 |
| <i>Staphylococcus haemolyticus</i> | 1 | - | - | 1 | - | 1 |
| <i>Staphylococcus epidermidis</i> | 2 | - | - | 2 | - | 2 |

S= Susceptible; I= Intermediate; R= Resistance.

milking. Generally, *S. aureus* has been designated as a causative agent of both clinical and subclinical mastitis.

The isolation rate of *S. agalactiae* (12.3%) in this finding coincides with that of Bitew et al. (2010) at Bahir Dar who reported 13.9%. However, the finding was higher than the 4% report by Lakew et al. (2009) from Asella, and 6.4% isolation rate reported by Sori et al. (2005) in and around Sebeta town.

E. coli was identified from 5% of the samples in this study and this proportion was lower than reports by Sori et al. (2005), Mekebib et al. (2009), Bitew et al. (2010) who reported an isolation rate of 26.57, 43.13 and 20.3%, respectively. This lower isolation rate of environmental mastitis causal agents might be partly associated with effective and good sanitation of the barns with immediate removal of feces practices. Moreover, the proportion of *Micrococcus* species in this study was lower than the finding of Mekonnen et al. (2005) and Bedada and Hiko (2011), who reported 10.2 and 5.6%, respectively. It was also reported that *Micrococcus* species causes mastitis only occasionally.

Mixed (fungal and bacterial) and fungal infection alone in this study represented 56 and 2% respectively. The overall 56% mixed fungal infection were comparable with

the result of Pachauri et al. (2013) who found 64%. However, this was lower than the results of Al-Ameed (2013) in Iraq who reported 80% and was higher than the 13% fungal mastitis prevalence reported by Sukumar and James (2012). This might be due to unhygienic condition of the animal sheds and high humidity along with favorable environmental conditions supporting growth of fungal spores. Hence favorable conditions increase the chances of fungal spore to enter into the udder which provide suitable environment to these fungi (Williamson and Di Menna, 2007). Under immunosuppressive conditions, the dynamics of microorganisms may be disrupted, and the fungi together with the other microorganisms are able to overcome the udder defense mechanisms.

The overall isolation rate of yeast from the current study was 38.18% of which *Candida* species accounted for 11.8%, *Yarrowia lipolytica* for 10.9%, *Rhodotorula* species for 3.2%, *Rhodospiridium diobovatum* (2.3%), *Galactomyces geotrichum* (3.6%), *Geotrichum terrestris* (1.4%), *Trichosporon species* (3.2%) and *Saccharomyces species* (1.8%). This isolation rate was lower than that reported by Andreia et al. (2008) on *Candida* (37.9%), *Cryptococcus* (10.3%) and *Rhodotorula* (10.3%). Although

the distribution of *Candida* species shows diversity in several countries, it is important to note the increase in number of mammary gland infections caused by *Candida* species in the recent years (Krukowski et al., 2001). Filamentous fungi was isolated from 20% of the tested samples, the isolated fungal species were *Aspergillus* (6.8%), *Mucor* (5.9%), *Penicillium* (3.6%) and *Fusarium* (3.6%). The isolation rate of *Aspergillus* species was lower than the 38% reported by Mdegela et al. (2005) and Blowey and Edmondson (2010). The management practices adopted on dairy cows, like discarding first few strips of milk on ground while milking of animals as well as during treatment of mastitic animals and reluctance to disinfect hand between milking by milkers may contribute as potent source of lateral transmission of fungal and yeast infections (Pachauri et al., 2013). There are also reports in which yeasts like *Candida* spp. utilizes nitrogen from penicillin and tetracycline antibiotics, antibiotic therapy leads to perturbation in udder homeostasis, inhibition of T cells and neutrophil activity and in consequence this may also stimulates yeast growth (Corti et al., 2003; Noris et al., 2007).

In the present study, *Staphylococcus* species were found resistant to penicillin G (93.1%) and oxytetracycline (79.3%) and this is comparable with many previous reports in the country. The resistance of *Staphylococcus* species to penicillin may be attributed to the production of beta lactamase, an enzyme that inactivates penicillin and closely related antibiotics. The development of antibiotic resistance probably is a result of repeated therapeutic use or indiscriminate use of these antibiotics (Jaims et al., 2002). The uses of antimicrobials have, overtime, increased the number of antimicrobial-resistant microbes globally, and any use of antimicrobial agents will to some extent facilitate the development of resistant strains (Williams, 2000). The majority of authors have noted the development of antimicrobial resistance by *Staphylococcus* species isolated from mastitis cases (Pitkala et al., 2004; Turutoglu et al., 2006; Pyorala and Taponen, 2009; Sori et al., 2011).

Conclusion

It could be concluded that bovine mastitis is a major challenge to the dairy producers in and around Sebeta towns. Large numbers of microorganism were isolated from milk of CMT positive cows with *Staphylococcus*, *Streptococcus*, *Candida* species and *Y. lipolytica* being the predominant. The demonstrated resistance pattern of *Staphylococcus* species to penicillin and oxytetracycline may alarm on the repeated use of these drugs for mastitis treatment in the country. Hence, comprehensive studies including molecular characteristics of drug resistance gene of *S. aureus* especially of methicillin-

resistant should be conducted in farm animals. In 2% of the cases, fungal species were identified as causes of mastitis, hence further investigation regarding their pathogenicity and contribution to bovine mastitis is needed.

ABBREVIATIONS

CI, Confidence interval; **CLSI**, Clinical Laboratory Standards Institute (CLSI); **CMT**, California mastitis test; **FAO**, Food and Agriculture Organization; **NAHDIC**, National Animal Health Diagnostic and Investigation Center; **OR**, odds ratio.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work is a part of sub-thematic research "Bovine mastitis: Udder morphometrical traits, common bacterial isolates, histopathological changes and predisposing factors to clinical and subclinical mastitis in local zebu and crossbreed dairy cattle in central Ethiopia "RD/LT-038/15" for which the investigators have received ethical clearance referenced with **VM/ERC/005/08/2015** from ethical clearance and animal welfare committee of Addis Ababa University college of Veterinary Medicine and Agriculture. After briefing the purpose of the study consent was requested from all participating dairy farm owners for collecting samples. All the procedures used were non invasive and in addition all the results were communicated to animal owners.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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