Bacterial identification and antimicrobial susceptibility of subclinical mastitis causing bacteria from goats in Aba’lla district, Afar, North-Eastern Ethiopia

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ABSTRACT
Subclinical mastitis is well known to cause huge economic losses in the dairy production. In the Afar region of Ethiopia goats are among the most important animals used for milk and meat production. The present study was conducted from February to June, 2014 to estimate prevalence of subclinical mastitis in goats using California Mastitis Test (CMT), to isolate major bacterial pathogens causing mastitis and to establish antimicrobial sensitivity for bacterial isolates. A total of 228 lactating goats from Aba’lla district, Afar region were examined. The prevalence of subclinical mastitis was 20.6% (47/228). Age showed statistically significant association with the prevalence of subclinical mastitis (p = 0.009). However, parity number and length of lactation did not show statistically significant relation with the prevalence of subclinical mastitis in goats. Through bacteriological examinations six different isolates from the CMT positive milk samples were S. aureus (11/47, 23.4%), E. coli (10/47, 21.3%), Streptococcus (9/47, 19.1%), Salmonella (8/47, 17.0%), Staphylococcus (5/47, 10.6%) and others (4/47, 8.5%). Antibiotic susceptibility of the selected isolates was investigated by disk diffusion method. High resistance of Staphylococcus species to penicillin G followed by ampicillin was observed. Streptococci were highly resistant to ampicillin, norfloxacin, ciprofloxacin and clindamycin. E.coli were resistant to clindamycin, whereas salmonella were found to be highly resistant to kanamycin, ampicillin and erythromycin. Some of the organisms were found to be multidrug resistant.

Keywords: Afar region, Antibiotic, Mastitis, Resistance

Introduction
Small ruminants play an important role in the nutrition and income of people worldwide. It has been estimated that there are more than 460 million goats worldwide producing about 4.50 million tons of milk and 1.20 million tons of meat annually. Small ruminants in Africa produce only 14.0% of the world’s milk [2, 23]. Ethiopia has the leading livestock population in Africa and the animal population census estimates the sheep and goat population of the country 25.5 million and 24.15 million, respectively [5]. Despite the large population of sheep and goats in Ethiopia, the economic benefits remain marginal due to prevailing disease, poor nutrition, poor animal production system, reproductive inefficiency, management constraints, and general lack of veterinary care. Among these factors, diseases have a major impact in morbidity and mortality rates with annual losses as high as 30-50% of the total value of livestock product of Ethiopia [22].

The major income of dairy goats and sheep comes from milk production, which is mainly processed into fermented product and cheese. Thus factors influencing milk quantity
and quality, such as subclinical mastitis, have a significant effect on economic losses to the farmer. In recent studies it was demonstrated that cured yield and its properties were damaged by subclinical mastitis as well, thus posing a secondary loss to the dairy industry [10, 11, 6]. Compared to developed countries, mastitis is a major problem in the dairy industry of developing countries like Ethiopia. Mastitis has both an extreme zoonotic and economic importance. It is the cause of multiple hazardous effects on human health and animal production [8].

Sub-clinical mastitis in small ruminants of Africa causes a significant impact of the production and productivity of dairy goats and sheep and this is associated with the absence of clinical signs to be detected by the clinicians or farmers [9]. A study conducted in Tigray region of Ethiopia identified Streptococcus species, S. aureus, coagulase negative Staphilococcus species and E. coli as the major causative agents of mastitis [6].

Currently, the other major concern to human health is the issue of antimicrobial resistance due to indiscriminate use of antibiotics in livestock production in developing countries. In Ethiopia, the major antibiotics used for treatment of mastitis include penicillins, streptomycins, gentamycin and oxytetracycline. The indiscriminate use of these antibiotics leads to antibiotic resistance. This gap of information is higher in Afar region in spite of the existence of huge number of goats; and this limitation can have a good opportunity for the transmission of different zoonotic diseases. Therefore, the objectives of the present study were to assess subclinical mastitis in lactating goats, identifying the major bacteria that cause subclinical mastitis in goats in Aba’alla district, Afar region. Furthermore, the antimicrobial susceptibility pattern of the isolated bacterial pathogens from goat milk was determined.

Materials and methods

STUDY AREA AND ANIMALS

The study was conducted in Aba’alla district (Formerly called Shekhet) in the northern part of the Afar National Regional State, northeastern Ethiopia. The study area lies approximately between 13°15’ and 13°30’ latitude and 39°39’ and 39°55’ longitude. The distance from Mekelle city is about 55 Km. The average elevation of area is approximately 1500 m.a.s.l. It is characterized by semi-arid type of climate receiving bimodal rainfall. The highest peak, mount Mussa-Alle is just 2063 m.a.s.l. The temperature of the region varies from 25°C during the rainy season (September-March) to 48°C during the dry season (March-September). In the present study 228 lactating Afar breeds of goats from 98 livestock owners were included). Thus taking the previous prevalence of 18.3% from a study conducted by Gebrewahid et al. [6] in Tigray, the calculated sample size was 228 [21].

STUDY DESIGN

A cross sectional study was conducted from February, 2014 to June, 2014 in Aba’alla district, the Afar National Regional State.

MILK SAMPLE COLLECTION

Milk samples were collected according to the National Mastitis Council [16]. After the halves have been washed with tap water, soap and dried the teat ends were swabbed with cotton soaked in 70% ethyl alcohol. After discarding the first three milking streams, approximately 5-10 ml of milk was collected aseptically from each lactating goat and placed in sterile test tube. The milk samples were transported on ice to Mekelle University, College of Veterinary Medicine Microbiology laboratory where they were immediately cultured or stored at 4°C for a maximum of 48 h until cultured on standard bacteriological media.

CALIFORNIA MASTITIS TEST (CMT) AND IDENTIFICATION OF BACTERIA

Milk samples from each halves were tested for subclinical mastitis using CMT. This screening test was conducted according to the standard procedure [17]. The CMT positive milk samples were subjected to bacteriological examination according to the standard protocols with minor modifications [16, 17, 14]. A loop full of milk sample was streaked on blood agar base (Oxoid, UK) enriched with 5-10% defibrinated sheep blood, and then sub-cultured on selective media for different bacteria using the quadrant streaking method; Mannitol Salt Agar for Staphylococcus, Eosine Methylene Blue Agar for E.coli, Salmonella Shigella Agar (SSA) for Salmonella spp and Edwards medium for Streptococci spp. In addition, the samples were also cultured on MacConkey Agar for differentiation. All the plates were incubated aerobically at 37°C for 24 – 48h. The plates were examined for gross colony morphology, pigmentation and hemolytic characteristics at 24-48h. For further identification of the organisms different biochemical tests (Gram reaction, the catalase test, haemolysis, O-F test, CAMP test, IMViC tests, TSI test, mannitol and maltose fermentation) were conducted [17].

ANTIMICROBIAL SUSCEPTIBILITY TEST

Antimicrobial susceptibility test was conducted on randomly selected S. aureus, Streptococcus, E. coli, and Salmonella isolates and the susceptibility test was conducted using Kirby-Bauer disk diffusion method [17]. About fifteen antimicrobials such as Cefoxillin (30µg), Norfloxacin (10µg), Chloramphenicol (5µg), Ciprofloxacin (5µg), Ampicillin (10µg), Penicillin (10µg), Gentamicin (10µg), Polymyxin-B, Clindamycin, Erythromycin, Sulphamethizole, Streptomycin, Kanamycin, Tetracycline and Vancomycin (Oxoid, Hampshire, England) were selected from main class.
of antimicrobials and investigated for sensitivity testing. The antibiotic disks were applied on the surface of the inoculated agar plates using aseptic technique. The inhibition zone diameters were measured to the nearest millimeter and strains were characterized as susceptible (S), intermediate (I) or resistant (R) [14].

DATA MANAGEMENT AND STATISTICAL ANALYSIS

The data was properly gathered and recorded; age, parity and lactation stage were the variables recorded. The prevalence of subclinical mastitis in the lactating goats was determined as the proportion of animals that showed CMT positivity out of the total examined. The effect of the above variables on the prevalence of the disease was determined using chi-square ($\chi^2$) test through SPSS (Social packaging statistical system).

Result

PREVALENCE OF SUBCLINICAL MASTITIS AND ITS ASSOCIATION WITH RISK FACTORS

Out of the total of 228 lactating goats from the study site, 47 of them (20.6%) showed CMT positive result.

During the study period, certain potential risk factors such as age, parity and length of lactation were taken in to consideration to see the integration with the occurrence of subclinical mastitis in lactating goats in the study area. Among the risk factors; age ($p = 0.009$), showed statistically significant association with the occurrence of subclinical mastitis in goats. Accordingly, higher rate of prevalence was recorded in goats older than 4 years. However, there was no statistically significant association between risk factors such as length of lactation ($p = 0.215$) and parity ($p = 0.30$) (Table I).

<table>
<thead>
<tr>
<th>Risk factor group</th>
<th>No. animal Examined</th>
<th>CMT Positive (No./%)</th>
<th>$\chi^2$ - value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4 year</td>
<td>119</td>
<td>17 (14.3%)</td>
<td>6.858</td>
<td>0.009</td>
</tr>
<tr>
<td>&gt; 4 year</td>
<td>109</td>
<td>31 (28.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation period in months</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1-3</td>
<td>133</td>
<td>23 (17.3%)</td>
<td>3.072</td>
<td>0.215</td>
</tr>
<tr>
<td>3-5</td>
<td>65</td>
<td>16 (24.6%)</td>
<td></td>
<td></td>
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<tr>
<td>&gt;5</td>
<td>30</td>
<td>9 (30.0%)</td>
<td></td>
<td></td>
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<tr>
<td>Parity by Number</td>
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<td></td>
</tr>
<tr>
<td>1-3</td>
<td>119</td>
<td>17 (14.3%)</td>
<td>6.994</td>
<td>0.30</td>
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<tr>
<td>3-5</td>
<td>90</td>
<td>25 (27.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>19</td>
<td>6 (31.6%)</td>
<td></td>
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</tr>
</tbody>
</table>

Table I: Prevalence and association of risk factors with the prevalence of subclinical mastitis

PREVALENCE OF BACTERIA ISOLATED FROM MILK SAMPLES

Out of 47 CMT positive milk samples cultured for bacteriological examination 11 (23.4%), 10 (21.3%), 9 (19.1%), 8 (17.0%), 5 (10.6%) and 4 (8.5%) were S. aureus, E.coli, Streptococcus, Salmonella, Staphylococcus other than S. aureus and other gram negative bacteria, respectively.

ANTIMICROBIAL SUSCEPTIBILITY RESULT OF BACTERIAL ISOLATES

The antimicrobial resistance profile of the bacterial isolates from milk samples of goats is summarized in Figure 1. Staphylococcus species showed resistance to antibiotics like Penicillin G (81.8%), Ampicillin (90.9%), Cefoxitin (63.6%), Ciprofloxacin (90.9%), Clindamycin (72.7%) and Vancomycin (100%). Streptococcus species showed 100% resistance to ampicillin, ciprofloxacin, norfloxacin and clindamycin. Whereas E.coli organisms showed 70%, 80% and 60% resistance to ampicillin, clindamycin and erythromycin, respectively and salmonella species were 75% resistance to ampicillin, kanamycin and erythromycin and 100% to clindamycin. Table II summarizes the multidrug resistance pattern of bacterial species isolated from milk sample of goats. All the bacterial isolates showed a multidrug resistance to at least two antibiotics. Out of the 11 S. aureus, 45.5% of them showed multidrug resistance to four antibiotics and 80% of E.coli organisms showed resistance to three antibiotics used.
SUBCLINICAL MASTITIS IN GOATS IN ETHIOPIA

Discussion

According to the present study lactating goats were highly infected with subclinical mastitis with an overall prevalence rate of 20.6% which is in close agreement with previous finding of other researchers; 18.3% in Tigray North Ethiopia by Gebrewahid et. al. [6] and 15.5% in Borana, South Ethiopia by Megersa et al. [12]. However, the prevalence rate reported in the present study was higher than the prevalence (9.8%) reported by Ndegwa et al. [15] in Kenya and lower than some previous reports in Ethiopia (40.9%) by Assefa et al. [3], in Oromiya region, and in Tanzania (51.5% by Swai et al. [20], 76.7% by Mibilu [13]). The higher prevalence of both contagious and environmental pathogens could be associated with the poor sanitation due to contamination with waste products like faces and urine. Such contaminated teats and udder could be a good environment for the growth of variety of microorganisms [17].

In the present study, there was higher linkage between the age of dairy goats and prevalence of subclinical mastitis. However, there was no statistically significant association between the prevalence of the diseases and factors like stage of lactation and parity. The possible reason for this could be some of the lactating goats may be aged without increasing their parity and this could also be associated with the inefficiency in the reproductive potential of these goats. Several studies and different literature indicate the increased prevalence of the disease with increase in age and an increase in the parity of goats. The variation in the prevalence of subclinical mastitis in the different studies could be due to the variation in breed of goats, husbandry practice and agro-
climatic conditions. Moreover, the approach used by the different researchers could be a reason for the variation in the prevalence of the disease.

The high prevalence of *Staphylococcus aureus* followed by *Escherichia coli* in this study is in accordance with other workers who found higher prevalence of these organisms from milk samples positive for dairy cow mastitis [19, 1, 7]. These findings and other research works justify that *S. aureus* is the most important and prevalent mastitis-causing organism globally, including Ethiopia. The high prevalence of *S. aureus* points to poor milking time hygiene as this pathogen is mainly spread during milking via milkers’ hands and in Ethiopia, mostly hand milking is practiced [4]. The higher prevalence of *E. coli* could be linked to the poor hygienic practices in the dairy environment.

This may be attributed to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without a mastitis control program [6, 18, 7].

The antimicrobial susceptibility tests carried out in this study indicated for the high resistance of bacterial organisms to different antibiotics. Some studies conducted in the country also justify for an increasing trend of antibiotic resistance [7]. The probable justification for the higher resistance of bacterial organisms to the existing antibiotics could be due indiscriminate and prolonged use of antibiotics by individual farmers without prescription of professionals.

The present study indicated considerable prevalence of subclinical mastitis in lactating goats in Aba’alla district, Afar National Regional State with the isolation of major pathogenic microorganisms from subclinical mastitis. The reported prevalence rate could be very important from the economic loss and public health point of view to the pastoral communities. The presence of antibiotic and multidrug resistant bacterial strains is also reported as a serious problem. Therefore, the responsible authorities should take the necessary actions to control the prevalence of the disease and also take measures on the indiscriminate and prolonged use of antibiotics in the study area in particular. It is also important to have legislations and guidelines on the use of antibiotics at country level.

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**References**


