Isolation and Identification of Major Bacterial Pathogen from Clinical Mastitis Cow Raw Milk in Addis Ababa, Ethiopia

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Abstract: An investigation was carried out cross-sectional to determine the prevalence of commonly occurring bovine mastitis pathogens at Addis Ababa Ethiopia. Samples were mainly drown from dairy caws that become clinically ill during clinical observation, which comes for ambulatory service to the Addis Ababa Sholla Veterinary Laboratory. A total of 102 isolates were studied from bacterial culture obtained from the bovine milk samples collected at the clinic in the period from December, 2011 to April, 2012. The most common microbial isolates were Streptococcus species (16.7%), Staphylococcus species (53.9%), Escherichia coli (18.6%)/Klebsiella species (3.9%) and mixed infection (6.9%). There was a significant association (p>0.05) between quarter level prevalence with the occurrence of pathogenic microorganism, which is isolated from mastitis milk. The associations of body condition with pathogens were significant (p<0.05) in Streptococcus intermedius, where as other isolates have a non significant difference (p>0.05%) with the association of animal body condition, all animals (poor, good and moderate) were equally exposed and harbored the disease. The bacterial isolates were S. aureus, coagulase negative staphylococci, (S. intermedius, S.hyicus), E.coli, Klebsiela and streptococcus species. S. aureus was the predominant isolate accounting for 45.1% followed by E. coli (18.6%) and streptococcus species (16.7%). There was a significant association of lactation period with streptococcal infection (p<0.05). Milk is one of the main components of the diet of the rural society as well as urban and pre-urban area of the study area. It is consumed in the raw state by community. The presence and consumption of such milk may constitute a public health hazard in addition to the reduced milk quality due to mastitis in cow.

Key words: Mastitis - Bovine - Pathogens - Addis Ababa - Rural

INTRODUCTION

Ethiopia has an extensive livestock resource, which constitutes a valuable economic role for the nation. However due to various reasons the country does not benefit from this enormous wealth in terms of production such as milk, meat etc. Although inclusion of milk in the diet is often not commonly available to the public. This may be due to the low amount of milk and inadequate milk preservation. Only 20% of the milk produced from animals for human consumption occurs in tropical region, even though these parts of the world have 74% of the human population and 65% of the bovine population [1].

The World Bank in 1993 has estimated the demand for milk and dairy products in sub-Saharan Africa will increase by 5.5 million tons in the year 2025, an annual growth rate of 4%, questioning the supply side. The expansion of milk output in sub-Saharan Africa has not kept pace with the increase in human population, the rates of increase of which are 1.4 % and 2.9% respectively. In Ethiopia, based on a study conducted by Ibrahim [2] from 1975 to 1987, the annual growth rate of total output of fresh cow milk is negligible. Hence, meeting the milk requirements of the growing human population is great concern [1].

The factors which limit milk production include disease, marketing, husbandry and genetics. Among the disease mastitis contributes directly to the low milk production. Mastitis, an inflammation of the mammary gland is a complex disease that generally involves interplay between management practices and having different causes, different degree of intensity and

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variation in duration and the residual effects [3]. The disease in dairy herd is of great economic importance due to a reduction in the milk yield, the change in milk quality, the possibility of permanent change to one or more quarter, or even to the entire udder and death of the cow as well as loss due to increased premature culling rate and cost of treatment. Mastitis has also an effect on the health and wellbeing of the calves [4].

Infectious agents particularly various species of bacteria are important etiologic agents of mastitis. The prevalence of different species varies geographically, temporally and also due to control measures adopted in herds. In addition different species cause different types of mastitis. Mastitis is one of the complex disease of dairy cows that generally involve inter play between management practice and infectious agent having different cause, degree of intensity and various in duration and effect. Pathogenic microorganisms that most frequently cause mastitis can be divided in to two groups based on their source: environmental pathogens and contagious pathogens. Contagious Pathogens are well adapted to survival and grow in the mammary gland and frequently cause infection lasting for week, month, or even years [5].

Mastitis not only brings huge economic losses of dairy cow production, but also do harm to dairy industry, public health and food safety. The pathogen of cow mastitis which is caused mainly by bacterium is complex [3]. The Safety of milk with respect to food born disease is a great concern around world this is especially true in developing countries like Ethiopia where the production of milk often take place under unsanitary conditions and consumption of raw milk which is typically produced in small dairy farm under unsatisfactory hygienic conditions is a common practice [6]. The production of milk is a great concern around the world this is especially true in developing countries like Ethiopia where the production of milk often take place under unsanitary conditions and consumption of raw milk which is typically produced in small dairy farm under unsatisfactory hygienic conditions is a common practice [6]. The aims of the present study were to isolate and identify major bacterial species that causes clinical mastitis and to elucidate risk factors associated with it and to assess the knowledge-attitude-practice (KAP) among dairy owners towards clinical mastitis.

MATERIALS AND METHODS

Study Animal: Privately owns small holder dairy farms and state dairy farms were involved in the study population. The study animals were dairy cows, cross breed of Holstein Friesian cattle at different age category. Most of cross breeds found in the city were kept intensively by commercial dairy farm and by small holder.

Study Design: A cross-sectional study and laboratory isolation of the bacteria were undertaken from October 2011 to April 2012 on dairy farms in Addis Ababa, Ethiopia. Cows were examined directly at quarter level for clinical manifestations.

Sampling and Test Procedure: Data regarding the different potential risk factors (age, parity, lactating stage and body condition) were collected from clinically ill cow for 102 lactating cows during clinical examination of the study period.

Milk Sample Collection: Raw milk samples were collected from clinically infected teat quarters of the cows. During sampling, the sample was collected aseptically and put in to sterile screw capped bottle and kept in an ice box containing ice packs and taken immediately to microbiology laboratory for bacteriological analysis. Then the sample was stored over night in refrigerator at 4°C until analyzed in the next day. Isolation and identification of bacteria was done according to the techniques recommended by Quinn et al. [7].

Bacteriological Examination of Milk Sample: Bacterial examination was done according to the National Mastitis Council, NMC [8]. Milk Samples which are kept in refrigerator overnight at 4°C for 24 hr. 0.1ml of the pre enriched broth of various dilution were seeded aseptically onto sterile nutrient agar and incubated at 37°C for 24-48hr. Once a pure culture was obtained, the results from a few comparatively simple tests can often identify the bacterium to a genus level. The first test which was done was Gram stain. A Gram stained smear from the culture establish the Gram reaction (Gram positive or Gram negative) and cellular morphology. After the bacteria were categorized as Gram positive and negative, the suspected culture was sub cultured in to different media depending on their Gram stain properties. Gram negative bacteria were sub cultured in to MacConkey agar, where as Gram positive bacteria were sub cultured in to nutrient agar, mannitol agar and blood agar. The suspected colonies were identified according to Quinn et al. [7].

Data management and Analysis: The collected data included the, quarter, parity, lactation stage and body condition were recorded depending on clinical inspection. The data were entered in Microsoft Excel sheet and
analyzed by using SPSS version 17.1 software and 95% was taken for significance level of the result. The Chi-square(x2) test was applied to test the existence of association between the pathogen and the risk factors and clinical mastitis with risk factors.

RESULTS

Prevalence: Isolation and identification of major bacterial species were carried out on milk from clinical mastitis cow by using primary and secondary biochemical tests. The result obtained from this study showed that out of the 102 samples collected and processed 102 (100%) was positive for cultural isolation of bacterial species.

From the total number (n=102) of clinically infected dairy cow, 46 (45.1%) of were infected with Staphylococcus aureus, whereas 3 (2.9%) of were infected with Staphylococcus intermedius, 6 (5.9%) were infected with Staphylococcus hyicus, 19 (18.6%) of were infected with E. coli, 4 (3.9%) were infected with Klebsiella, 17 (16.7%) of which were infected with Streptococcus species and 7 (6.9%) of which were infected by mixed bacterial infection.

Risk Factors Affecting Prevalence of Clinical Mastitis:
The above table (Table 1) shows that; there is a significance difference between quarter with the pathogenic micro organism in case of mixed infection between quarter milk sample and the occurrence of pathogenic micro organism (i.e. p>0.05) for other. However, the highest prevalence has been recorded in Staphylococcus aureus 25(56.8%) from all quarter milk prevalence is recorded in mixed infection 1(2.3%) from all quarter milk sample.

The above table (Table 2) shows that; there is significance difference between body condition with the pathogenic micro organism in case of Staphylococcus intermedius infection (i.e. P<0.05). There is no significance difference between quarter milk sample and the occurrence of pathogenic organism (p>0.05) for others.

The highest prevalence has been recorded in S. aureus 8(47.1%) from poor body condition milk samples and the lowest prevalence was recorded in Staphylococcus intermedius 0(0%), Klebsiella 0(0) and Staphylococcus hyicus 0(0%) from poor body condition milk sample. The highest prevalence has been recorded in Staphylococcus aureus 30(47.6%) from moderate body condition milk sample and the lowest prevalence was recorded in Staphylococcus intermedius 0(0%) from moderate body condition milk sample. The highest prevalence has been recorded in Staphylococcus aureus 8(36.4%) from good body condition milk sample and the lowest prevalence was recorded in Staphylococcus hyicus 1(4.5%) and klebsiela 1(4.5%) from good body condition milk sample.

Table 1: Prevalence of clinical mastitis in different quarters of the infected teat.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>All (102)</th>
<th>Fr (25%)</th>
<th>Fl (44%)</th>
<th>Hr (6)</th>
<th>hl (4)</th>
<th>mix (1)</th>
<th>total (46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>25 (56.8%)</td>
<td>3 (30%)</td>
<td>0 (0%)</td>
<td>2 (50%)</td>
<td>4 (25%)</td>
<td>12(52.2%)</td>
<td>46(45.1%)</td>
</tr>
<tr>
<td>S. intermedius</td>
<td>1 (2.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1(4.3%)</td>
<td>3(2.9%)</td>
</tr>
<tr>
<td>S. hyicus</td>
<td>2 (4.5%)</td>
<td>0 (0%)</td>
<td>1 (20%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2(8.7%)</td>
<td>6(5.9%)</td>
</tr>
<tr>
<td>E. coli</td>
<td>1 (3.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>1 (3.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>7 (15.9%)</td>
<td>3 (60%)</td>
<td>3 (60%)</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
<td>4 (36.4%)</td>
<td>6 (11.8%)</td>
</tr>
<tr>
<td>Mixed infection</td>
<td>1 (2.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
<td>4 (25%)</td>
<td>1 (4.3%)</td>
<td>7 (6.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>44 (43.13%)</td>
<td>11 (10.7%)</td>
<td>5 (4.9%)</td>
<td>4 (3.9%)</td>
<td>16 (15.6%)</td>
<td>23 (22.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Prevalence of clinical mastitis in different levels of body condition

<table>
<thead>
<tr>
<th>Body condition</th>
<th>Poor (8)</th>
<th>Moderate (30)</th>
<th>Good (8)</th>
<th>Total (46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>8(47.1%)</td>
<td>30(47.6%)</td>
<td>8(36.4%)</td>
<td>46(45.1%)</td>
</tr>
<tr>
<td>S. intermedius</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>3(13.6)</td>
<td>3(2.9%)</td>
</tr>
<tr>
<td>S. hyicus</td>
<td>0(0%)</td>
<td>5(7.9%)</td>
<td>1(4.5%)</td>
<td>6(5.5%)</td>
</tr>
<tr>
<td>E. coli</td>
<td>3(17.6%)</td>
<td>11(17.5%)</td>
<td>5(22.7%)</td>
<td>19(18.6%)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>0(0%)</td>
<td>3(4.8%)</td>
<td>1(4.5%)</td>
<td>4(3.9%)</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>5(29.4%)</td>
<td>10(15.9%)</td>
<td>2(9.1%)</td>
<td>17(16.7%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>1(5.9%)</td>
<td>4(6.3%)</td>
<td>2(9.1%)</td>
<td>7(6.9%)</td>
</tr>
</tbody>
</table>

Significant at <0.05

Table 1: Prevalence of clinical mastitis in different quarters of the infected teat.

Table 2: prevalence of clinical mastitis in different levels of body condition
Table 3: Prevalence of clinical mastitis in relation to parity number.

<table>
<thead>
<tr>
<th>Parity</th>
<th>S. aureus</th>
<th>S. intermidius</th>
<th>S. hicius</th>
<th>E. coli</th>
<th>Klebsiella</th>
<th>Streptococcus</th>
<th>Mixed infection</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>11(40.7%)</td>
<td>1(3.7%)</td>
<td>1(3.7%)</td>
<td>3(11.1%)</td>
<td>4(14.8%)</td>
<td>2(6.25%)</td>
<td>5(18.5%)</td>
<td>27(26.4%)</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>34(46.6%)</td>
<td>2(2.7%)</td>
<td>5(6.8%)</td>
<td>16(21.9%)</td>
<td>0(0%)</td>
<td>14(19.2%)</td>
<td>2(2.7)</td>
<td>73(71.5%)</td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>1(50%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>2(1.9%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>46(45.1)</td>
<td>3(2.9%)</td>
<td>6(5.9%)</td>
<td>19(18.6%)</td>
<td>4(3.9%)</td>
<td>17(16.7%)</td>
<td>7(6.9%)</td>
<td>102(100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Prevalence of clinical mastitis in relation to different lactation stages.

<table>
<thead>
<tr>
<th>Lactation</th>
<th>S. aureus</th>
<th>S. intermidius</th>
<th>S. hicius</th>
<th>E. coli</th>
<th>Klebsiella</th>
<th>Streptococcus</th>
<th>Mixed</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3</td>
<td>9(30%)</td>
<td>1(1.7%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>9(30%)</td>
<td>2(6.7%)</td>
<td>30(29.41%)</td>
<td>0.123</td>
</tr>
<tr>
<td>6-Mar</td>
<td>30(90%)</td>
<td>1(1.7%)</td>
<td>5(18.5%)</td>
<td>1(3.3%)</td>
<td>3(5%)</td>
<td>8(26.7%)</td>
<td>4(6.7%)</td>
<td>60(58.8%)</td>
<td>0.797</td>
</tr>
<tr>
<td>&gt;6</td>
<td>7(58.3)</td>
<td>2(16.7%)</td>
<td>1(8.3%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>8(26.7%)</td>
<td>4(6.7%)</td>
<td>12(11.7%)</td>
<td>0.035</td>
</tr>
<tr>
<td>Total</td>
<td>46(45.1)</td>
<td>19(18.6%)</td>
<td>6(5.9%)</td>
<td>3(2.9%)</td>
<td>4(3.9%)</td>
<td>17(16.7%)</td>
<td>7(6.9%)</td>
<td>102(100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 shows that there was a significant difference between parity and pathogenic micro organism in case of klebsiella and mixed species infection (i.e. p<0.05), whereas for other pathogens there is no significant difference between pathogen and parity. However, the highest prevalence has been recorded in *Staphylococcus hicius* infection, 11 (40.7%). In case of 1-2 No of parity milk sample and the lowest prevalence was recorded in *Staphylococcus intermidius* 1(3.7%) and *Staphylococcus aureus* 11 (40.7%). In case of 1-2 parity milk sample. Highest prevalence has been recorded in *Staphylococcus aureus* infection 34 (46.6%) from 3-4 parity milk sample and the lowest prevalence was recorded in **klebsiella** 0(0%) from 3-4 parity milk sample.

The highest prevalence has been recorded in *Staphylococcus aureus*, 1(50%) and *streptococcus species*, 1(50%) above 4 parity milk sample and the lowest prevalence was recorded in the rest of other micro organism 0(0%) from above 4 parity milk samples.

Table 4 shows that there is a significance difference between lactation and pathogenic micro organism in case of *streptococcus species* infection (p<0.05). Whereas for other pathogens there is nosignificance difference between pathogens and lactation stage.

The highest prevalence has been recorded in *Staphylococcus aureus* 9 (30%) and *streptococcus species* infection 9 (30%) in case of 0-3 month of lation stage milk sample and the lowest prevalence is recorded in *Staphylococcus hicius* 0(0%) in case of 0-3 month of lactation stage milk sample. The highest prevalence has been recorded in *Staphylococcus aureus* infection 30(50%) in case of 3-6 month of lactation and the lowest prevalence is recorded in *S. intermidius* 1(1.7%) from 3-6 month lactation stage milk sample.

Highest prevalence has been recorded in *Staphylococcus aureus* infection 7 (58.3) from above 6 month of lactation stag milk sample and the lowest prevalence is recorded in Klebsiella 0(0%) and *streptococcus species* 0(0%) from above 6 month lactation stage milk samples.

**DISCUSSION**

Milk samples were collected from all dairy cows that became clinically ill during clinical examination of the study period. A total of 102 milk samples were collected from clinically mastitis cow from small holder and state farm from Addis Ababa city. The result of the study showed that the important bacterial organism isolated in selected sites dairy farm and small holder owners within Addis Ababa city over a six month period from November 2011- April 2012, were *staphylococcus species*, *streptococcus species* and *Escherichia coli*, as has been reported in other study [5, 9 &10] as well as klebsiella.
Prevalence was recorded as, 45.1% for *S. aureus*, 2.9% for *S. intermedius*, 5.9% for *S. hyicus*, 18.6% for *E. coli*, 3.9% for klebsiella, 16.7% for *streptococcus* species and 6.9% for mixed infection.

The study revealed that the prevalence of clinical mastitis was significantly affected (p< 0.05) by different stage of lactation <3 (29.11%), 3-6 (58.8%), >6 (11.7%), early, mid and late lactation stage respectively, which was comparable with research of [11-13] that reported high prevalence rate of clinical mastitis of cow in mid lactation. However, Kerro and Tareke [14] reported higher prevalence in early stage of lactation which disagrees with prevalence was recorded. This was in agreement with the research of Biffa et al. [13] in South study prevalence of clinical mastitis was more common in cows all quarter milk sample (43.13%). This may be due to poor hygienic husbandry practice like poor personal hygiene because bacteria transmit from infected to uninfected quarter by the contaminated hands of the milker. Thus the milker's hands should be washed thoroughly with disinfected soaps before milking and teats should be cleaned and dried off before milking. In addition to this the infection may also result due to vigorous suckling by calves which are known to cause direct inflammatory reaction to the mammary gland, necrosis and abscess formation, which may lead to udder damage and or exposure to serious secondary infections [16]. It could also be due to traditional dairy husbandry practices whereby calves are kept away from their dam over a long period of time and are only allowed to suckle for a short period as well as inadequate milk supply which lead to calves sucking vigorously, inducing teat injuries and subsequent infection of the mammary gland [16].

This is supported by the work of Capuco et al. [17] who reported that partial removal of keratin from the teat canal compromise the ability of the teat to prevent passage of bacteria pathogens from the external environment into the mammary glands. The high prevalence recorded could as well be attributed to the poor milking hygiene practices such as lack of usage of disinfectant on udder, teat dipping and lack of instituting dry cow therapy. The lack of surveillance program for mastitis could also be a contributory factor [18].

The prevalence of mastitis for cow that gives birth 4 and above was 1.9%; this findings were significantly lower than the findings of other researchers like [19] who reported prevalence of 65% during last parity. In this study the medium the parity number the higher the prevalence was recorded. This was in agreement with the research of Biffa et al. [13] and Tesfaye [20]. This could be associated with the possibility of exposure to the infectious agent with increasing number of parity.

According to Erskine et al. [9] primiparous cows have more effective defense mechanism than multiparous cows. But the present study revealed a lower prevalence of mastitis 1.9% in cows that gave more than four births followed by 31.3 % in cows that gave 1-2, births and a higher (71.5 %) in cows that gave more than 3-4 births, in which occurrence of mastitis and parity was insignificant (p>0.05). This may be due to the influence of breed, management and agro ecological environment. In this study prevalence of clinical mastitis was more common in a cow having moderate body condition and the lowest prevalence was recorded in a cow having poor body condition. This finding is contradicted with the expected one because the cow supplied with well balanced diet have reasonable resistant to mastitis infection [21] but this may be due to the fact that most of the cows which were included in this study are reared under good husbandry practice in which the cow is provided with relatively well balanced diet and the cow staying most of its times at indoor environment.

Laboratory results indicated that from 102 milk samples subjected to bacteriological examination 46 (45.1%) were isolates of *S. aureus*. This indicates that this bacteria are the most prevalent bacteria from all bacterial species that were isolated from clinical mastitis milk. These findings are disagreed with the report of Bitew et al. [22] 20.3%, in Bahir Dar [23] 17.8%, Bishi [24] and Hussein [25], 9 and 10.6% in the same area with this study respectively. The findings of this study were significantly higher than the above indicated works. The relatively higher prevalence of *S. aureus* can most likely related to the wide distribution of the organism inside the mammary gland and on the skin of teat and udder [26]. *S. aureus* has adopted to survive in the udder...
and established chronic and subclinical infection [27]. The isolation of *staphylococcus aureus* is of public health significance since it is a commonly recovered pathogen in outbreaks of food poisoning due to milk and milk product [28].

Laboratory results indicated that the prevalence of *Staphylococcus intermedius*, *Staphylococcus hyicus* was 2.9 and 5.9 respectively. *Staphylococcus intermedius* can produce sub acute mastitis which is sensitive to antibiotics therapy [28]. Presence of this organism around the nipple induces the aggravation of leukocytes in that area which in turn prevent invasion of serious mastitis producing bacteria. *Staphylococcus hyicus* is believed to be coagulase negative non hemolytic bacteria that may produce pigment. It is rarely a causative agent of mastitis. Their presence in milk sample from clinical mastitis is less frequent, however being occurring as a part of normal flora all warm blooded animal they present in the milk [29].

Laboratory results indicated that the prevalence of *Escherichia coli* is 18.6%. But this finding is much lower than the finding of Iqbal et al. [30] (40.7%). The prevalence of *Escherichia coli* is presumably due to the fact that *E. coli* is the commonest environmental contaminants, which is closely associated with hygiene. It becomes pathogenic whenever the hygienic conditions of the animal or environment become poor. Moreover, the existence of high concentration of *E. coli* in milk also indicates the relatively poor quality of milk, related with substandard hygiene of the farm management, milk collection and processing system. The isolation of *E. coli* is of public health significance as this bacterium is known to cause serious gastrointestinal disorders in both young and adult humans [16].

Laboratory results indicated that, From 102 milk sample subjected to bacteriological examination the prevalence of *streptococcus species* is 16.7%. The isolation of *streptococcus* in this work is in agreement with that of Rehman et al.[31] who isolated 39 (20.4%) and 123 (15.4%) respectively from apparently healthy and mastitis cow in Kenya. The presence of *Streptococcus species* was also an indication of sub-standard dairy farming. The isolation of *strepptococcus species* is of public health significance as it causes various gastrointestinal upset ranging from abdominal pain to diarrhea [16].

Laboratory results indicated that the prevalence of Klebsiella is 3.9%. The presence of Klebsiella in milk sample is less frequent. However, being a coliform it may occur in milk samples probably as contaminants [16]. Laboratory results indicated that the prevalence of infection with more than one bacterial species is 6.9% which may occur as a secondary bacterial complication following to teat or udder injuries.

**CONCLUSIONS**

The present study was conducted on isolation and identification of major bacterial pathogen from clinically mastitis cow raw milk in Addis Ababa from November 2011- April 2012. And the prevalence of mastitis causing pathogens was; *S. aureus* (45.1%), *Staphylococcus (2.9%) intermedius*, *Staphylococcus hicius* (5.9%), *Escherichia coli*–18.6%, *Streptococcus species* (16.7%) and Klebsiella (3.9%).This indicates that mastitis caused by *S. aureus* is one of the major problems of dairy cows in milk production followed by *E.coli*. The distribution of these bacterial pathogens in the herd indicates the economic impact of the disease. Beside the disease has economic importance it also do harm the health and well being of human being. Therefore it requires proper diagnosis and treatment measures. Depending on the finding results from this work the following recommendations are forwarded.

- Dairy farmer should be informed about the hygienic method of milking cows.
- The professionals should apply different methods for prevention and control of the disease.
- The professionals should be informed on the public about the relevance of pasteurization of milk before consumption to avoid food born infection and intoxication.
- The governmental authority should work on the bacterial load of cow milk in our country and have to generate a standard.
- There is a need of further study on the virulence and behavior of different pathogenic micro organism that cause mastitis.

**Conflict of Interest:** We declare that we have no conflict of interest.

**REFERENCES**

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