Antimicrobial susceptibility profiles of mastitis isolates from cows in three major Ethiopian dairies

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INTRODUCTION

Bovine mastitis is a major cause of economic loss in dairy cattle worldwide. A routine part of its control involves administration of antibiotics by intra-mammary and/or systemic routes. However, a number of studies showed that bacterial isolates from clinical and sub-clinical cases of mastitis exhibit varying degree of susceptibility to antimicrobial drugs [1, 8, 10, 12]. Loss from mastitic cows treated with poorly active antibiotics was found to be over 4.5 times greater than that of mastitic cows treated with antibiotics of favourable response rate [7].

In Ethiopia, although some studies on bovine mastitis have been conducted in few farms in past years [2, 6, 16] and showed the prevalence rate ranging from 40% to 67%, information on the resistance pattern of major bacterial pathogens of mastitis in dairy farms is scarce. The present study was, therefore, undertaken to determine the resistance pattern of bovine mastitis bacteria to commonly used antimicrobials in the study farms.

MATERIALS AND METHODS

Milk samples collected from cows affected with clinical and sub-clinical (positively screened with California Mastitis Test) mastitis, from three dairy farms (Repi, Debre-Zeit and Modjo dairy farms) belonging to the Dairy Development Enterprise (DDE) of the Federal Government of Ethiopia, were cultured at the microbiology laboratory of the Faculty of Veterinary Medicine. The milk samples were examined bacteriologically following standard procedures [14, 15]. About 10 µL from each milk sample was streaked on to tryptose blood agar base enriched with 7% sheep blood (Oxoid, Unipath Ltd, Basingstoke, UK). Blood agar plates
were incubated aerobically at 37°C for up to 72 h. The plates were examined for growth, colony morphology and haemolytic characteristics at 24, 48 and 72 h after inoculation. Bacteria on culture positive plates were identified according to their Gram- stain reaction, colony morphology, haemolytic characteristics and catalase test. *Staphylococcus*, *Micrococcus* and *streptococcal* species were identified following standard methods [14, 15]. Gram-negative isolates were inoculated on to MacConkey’s agar (Oxoid, Unipath Ltd, Basingstoke, UK) and then evaluated by standard procedures [14, 15].

Sample size for antimicrobial resistance patterns was determined at 10% confidence interval and 95% confidence level by using a mathematical formula recommended for this purpose (www2.uta.edu/sswmindel/S6324/Class Materials/Sampling/sampsize).

The antimicrobial resistance patterns of the isolates were determined using the Kirby-Bauer disk diffusion technique [11, 14]. The disks were impregnated with the following antibiotics: tetracycline (5 µg), kanamycin (30 µg), erythromycin (15 µg), chloramphenicol (5 µg), streptomycin (10 µg) and ampicillin (2 µg) (Oxoid, Unipath Ltd, Basingstoke, UK). Ampicillin, streptomycin, erythromycin and oxytetracycline were commonly used antimicrobials for the treatment of mastitis in the study farms.

The zone of growth inhibition was reported as the diameter of the zone surrounding individual disks in which bacterial growth was absent. Those strains tested were scored as susceptible, intermediate or resistant following the recommended NCCLS interpretive standards [11]. An isolate was defined as resistant if it was resistant to at least one of the tested antimicrobial agents where as multiple drug resistance was defined as resistance to two or more antimicrobial drugs.

### Results

The results of bacteriological analysis are presented in Table I. From a total of 283 isolates *Staphylococcus* species represented 52% and *Streptococcus* species 25%. Other bacterial isolates, in their order of decreasing frequency, were *Micrococcus* spp., *Escherichia coli*, *Corynebacterium* spp., *Bacillus* spp., *Actinomyces pyogenes* and *Klebsiella pneumoniae*.

Antimicrobial susceptibility test was done on a total of 79 randomly selected isolates. The distribution and percentage of isolates tested for antibiotic susceptibility are shown in Table I.

Table II illustrates the antimicrobial susceptibility results of bacterial isolates for six antimicrobial agents tested. Due to the relatively small sample size, no separate analysis was done for clinical and sub clinical isolates. Moreover, as the isolates scored intermediate were very few in numbers they were included in the susceptible category. *Staphylococcus aureus* that represented 36% of the total isolates was found highly sensitive, in vitro, to kanamycin (100% susceptibility) and very resistant to ampicillin (only 17.6% susceptibility). Erythromycin and streptomycin were found active against this organism in only half of the strains. Coagulase negative staphylococci were also found more sensitive to kanamycin and tetracycline. The activity of ampicillin, streptomycin and chloramphenicol was, however, moderate (46.7% susceptibility) against the coagulase negative staphylococci. Next important isolate, *Streptococcus agalactiae* (representing 14% of the total isolates) was found susceptible in vitro at least half of the time to all antibiotics tested, with highest susceptibility (80% susceptibility) to kanamycin, erythromycin and chloramphenicol. *Streptococcus dysgalactiae* showed more than 50% resistance to half of the antibacterials tested and exhibited highest susceptibility (80% susceptibility) to kanamycin and ampicillin. *Streptococcus uberis* was most sensitive to chloramphenicol (100% susceptibility) and kanamycin (80% susceptibility) and least responsive against erythromycin (40% susceptibility). *Micrococcus* species representing 10% of the total isolates found poorly susceptible to chloramphenicol and streptomycin (40% susceptibility) and highly susceptible to kanamycin and erythromycin.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Number of isolates</th>
<th>Proportion of strains tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinical mastitis</td>
<td>Sub-clinical mastitis</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>13</td>
<td>91</td>
</tr>
<tr>
<td>coagulase negative staphylococci</td>
<td>10</td>
<td>34</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td><em>Streptococcus dysgalactiae</em></td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td><em>Streptococcus uberis</em></td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td><em>Micrococcus</em> spp.</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>Actinomyces pyogenes</em></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><em>Bacillus</em> spp.</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>71</strong></td>
<td><strong>212</strong></td>
</tr>
</tbody>
</table>

Table I. — Distribution of mastitis pathogens isolated from clinical and sub-clinical cases of mastitis in tree major Ethiopian dairies.
cin (100% susceptibility). *Escherichia coli* that represented around 4% of the total isolates was found highly resistant (only 16.7% susceptibility) to erythromycin and highly susceptible to chloramphenicol and kanamycin. *Actinomyces pyogenes* were more often susceptible to all antibacterials tested. *Corynebacterium* spp. were found resistant (33.3% susceptibility) to erythromycin and chloramphenicol. The proportion of *Bacillus* spp. isolates susceptibility to the antimicrobials was consistently higher than 60% with the exception of erythromycin. In general, it was found that for the bacterial isolates tested for antimicrobial susceptibility, kanamycin and tetracycline were the drugs more active while the majority of isolates were resistant to ampicillin and erythromycin.

### Discussion

An antimicrobial’s response rate may be qualified as poor when it cures less than or equal to 25% and said favorable when the response rate attains 75% or above [7]. The antimicrobial susceptibility tests carried out in this study indicated the existence of resistance against one or more antibiotics in some of the bacterial isolates. The *in vitro* antibiotic susceptibility of a pathogen may not necessarily indicate successfullness of therapy, but antibiotic resistance can be interpreted as high probability of treatment failure [9].

The average susceptibility (62.7%) of *Staphylococcus aureus* strains to all antimicrobials tested in this study is comparable to the existing reports [10, 17]. The poor inhibitory effect of ampicillin against *Staphylococcus aureus* strains (17% susceptibility) observed in this study is also in agreement with what was reported by MACKIE et al. [8]. The latter reported *Staphylococcus aureus* isolates sensitivities to ampicillin of 19 and 17 percent respectively from clinical and sub-clinical mastitis for the year 1986 in Northern Ireland. Other workers [4, 12], however, reported much lower level of resistance. According to OLIVEIRA et al. [12] there exists variation among countries regarding the antimicrobial susceptibility pattern of *Staphylococcus aureus* for ampicillin. Information on previous studies conducted within Ethiopia is not available to compare our test results. However, considering the high frequency of *Staphylococcus aureus* and routine use of ampicillin in the farms, the existing practice with regard to the choice and use of antibiotics can be questioned.

The antibiotic susceptibility test results of coagulase negative staphylococci isolates presented in Table II had a trend comparable with what was reported by MYLLYS et al. [10]. The latter reported that “coagulase negative staphylococci were more often susceptible to all antibacterials than *Staphylococcus aureus* isolates, but resistant coagulase negative staphylococci isolates were more often multi resistant than *Staphylococcus aureus* isolates”.

Among *Streptococci* spp. isolated, *Streptococcus dysgalactiae* showed considerable multi resistance to antimicrobials, a finding in contrast with previous reports [10]. However, the limited number of strains tested in this study demands cautiousness in the interpretation of our results. Results of this study regarding *Escherichia coli* susceptibility levels to ampicillin, tetracycline and erythromycin are relatively higher than the reports of BEZEK [1] and lower than that of ERSKINE et al. [4].

The present study demonstrated the existence of alarming level of resistance of frequently isolated mastitis bacteria to commonly used antimicrobial agents in the study farms; a result in accordance with reports from earlier works in other countries [3, 5] suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. It is, therefore, very important to implement a systematic application of an *in vitro* antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections. Meanwhile, due to limited sample size tested in the present study, additional studies involving larger sample size and dairy herds will have

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**Table II.** — Antimicrobial susceptibility of mastitis pathogens isolated from clinical and sub-clinical cases of mastitis in three major Ethiopian dairies.

Values within brackets indicate antimicrobial susceptibility in percentage, n: total number of strains tested, AMP = ampicillin, TET = tetracycline, ERY = erythromycin, CMP = chloramphenicol, K = kanamycin and S = streptomycin.
greater use to formulate guidelines with regard to the choice and use of antibiotics in both the treatment and prevention of intra-mammary infections.

References