Bacteriological Evaluation of Present Situation of Mastitis in Dairy Cows

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Abstract: The purpose of study was to surveying and identifying the bacterial causes of bovine mastitis. One hundred and twelve bovine milk samples from clinical, sub-clinical and apparently healthy cases were collected and were tested by CMT and SCC. The percentage of subclinical mastitis was 56.3% while that of clinical mastitis was 13.3%, these +ve samples were used for bacteriological culture to isolate the bacterial agents causing mastitis on different types of media. And then were identified using different API systems. The causative agents were either single pathogens like *E. coli* (25.5%), *S. aureus* (14.8%), CNS (12.7%) *St. agalactiae* (12.7%), *St. pyogens* (10.6%) *K. pneumoniae* (8.5%), *Salmonella* spp. (4.2%), *Proteus* species (4.2%), *Ps. aeruginosa* (4.2%) and *C. albicans* (2.1%) or mixed infection like *S. aureus* plus *E. coli* had the highest prevalent rate (17.8%) followed by *E. coli* plus *K. pneumoniae*, *S. aureus* plus *K. pneumoniae* and CNS plus *E. coli*, gave 14.2%. Then followed by *S. aureus* plus *St. agalactiae* plus *E. coli*, *S. aureus* plus *E. coli* plus *K. pneumoniae*, *St. agalactiae* plus *E. coli*, *St. pyogens* plus *E. coli* and CNS plus *K. pneumoniae* gave 7.1%. The lowest prevalent mixed infection was *Proteus* spp. plus *S. aureus* displayed 3.5%.

Key words: Mastitis - California Mastitis Test And Somatic Cell Count

INTRODUCTION

Mastitis means inflammation of the mammary gland and is characterized by physical, chemical, microbiological and cellular changes in milk as well as pathological changes in the udder [1]. Mastitis includes clinical and subclinical forms. Subclinical-mastitis is that form of mastitis where infection and inflammation occurred in the udder without observable or palpable external changes in the udder or in the secreted milk [2]. In clinical mastitis, however, abnormalities of the udder and milk are to be observed as changes in the milk such as flanks, clots and watery appearance as well as cardinal signs of inflammation on the udder or even atrophy of the tissue [3].

From the economic point of view, mastitis especially the-subclinical form causes extensive economic losses that include reduction of milk yield, changes in the milk composition and reduction in milk as well as shortens the productive life span of the affected animals. Also costs of drugs and veterinary services [4, 5]. In addition to economic impact and the bacterial contamination of the milk may render it unsuitable for human consumption [6].

Bovine mastitis mostly occurs due to bacterial, rickettsial and fungal invasion and infection as well as physical injury of the udder during the act of milking process. So, [7] recorded that machine milked cows show high incidence of mastitis than that of hand milked cows.

The microbial causes of mastitis have been categorized according to [2] to either contagious pathogens (e.g., *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae*) or environmental pathogens (e.g., *E. coli* and *Streptococcus uberis*). Regardless the fact that more than 135 different microbial species have been reported as a cause of bovine mastitis, the majority of infections are caused by staphylococci, streptococci and Gram negative bacilli.

More attention has been given to the diagnosis of clinical and subclinical mastitis using indirect tests, which depends upon the cellular interaction between reagent and certain protein factor in mastitis milk. These tests includes somatic cell count (SCC) [8] and California mastitis test (CMT) [9].

Several methods have been reported for detection of mastitis as isolation of the causative microorganism(s) which is the most accurate one. But, it is expensive and
time consuming. So, the need for a simple quite sensitive, rapid and reliable test sufficient to be applied on large scale of animals is therefore required [10]. The aim of our work to determined the bacteriological evaluation of Present Situation of Mastitis in Dairy Cows in Egypt that help to treatment and control of bovine mastitis.

**MATERIALS AND METHODS**

**Milk Samples:** A total number of 112 milk samples was collected from dairy cattle aseptically for bacteriological examination. The samples were collected from cases either atrophied quarter, dried or suffering from severe mastitis according to the procedures of Put the authors with each Ref. separate [10, 11].

A sample of 15-20 ml of milk was drawn in a clean sterile screw capped bottle then labeled. From each quarter, two milk samples were taken in a sterile tube. The milk samples were kept in an ice container till delivered to the laboratory. One of the two samples was examined for somatic cell count (SCC) (this sample was kept on formalin 10% if it will not examined for the somatic cell count at the same day). The second sample was subjected for bacteriological examination by incubating the sample in an incubator for 24 hours [10] then subjected for the bacteriological examination.

**California Mastitis Test (CMT):** It is a rapid, accurate, cow-side field test recommended by the American Public Health Association [12].

The CMT reagent reacts with DNA of epithelial and inflammatory cells present in the milk sample. CMT results were read immediately and were scored for each quarter depending on the thickness and amount of the gel formed, the CMT scores of '0' and trace (+) were taken as negative or normal whereas, CMT scores of 1+ (weak positive), 2+ (distinct positive) and 3+ (strong positive) were considered as indicators of subclinical mastitis. In the present study, a subclinical mastitis case was defined as an animal with at least one of the quarters with a CMT score of 1+.

**Somatic Cell Count (SCC):** The milk samples were examined for somatic cell count automatically using Bently Soma Count 150 (Bently, USA) according to Zecconi et al.[8] to detect any possible variation as well as bacteriological examination to correlate and investigate the possible associated causal agent, even the samples showed negative CMT were studied as a control.

**Bacteriological Examination:** Milk samples showed strong positive reactions in SCC and CMT were taken for bacteriological culture. Isolation and identification of bacterial agents causing mastitis was carried out [2, 13, 14].

**Isolation:** The milk samples were pre-incubated at 37°C for 18-24 hours, then a loopful from the samples was streaked onto 5% sheep blood agar, mannitol salt agar and MacConkey's agar plates, then incubated aerobically at 37°C for 24-48 hours. Suspected colonies were picked up, sub-cultured onto nutrient agar slants then incubated aerobically at 37°C for 24 hours.

**Identification of the Bacterial Isolates:** According to Quinn et al. [13], suspected colonies were examined for their gross appearance (morphological characteristics) including the colony size, shape, surface texture (rough or smooth), color of the colonies or the pigment production, the consistency (mucoid or non-mucoid), the hemolytic activity on the blood agar, type of hemolysis (α or β or γ type of hemolysis) and the metabolic activity on MacConkey agar (lactose fermenter (LF) or non-lactose fermenter (NLF)).

Suspected colonies were examined microscopically using Gram stained films before transferred onto slope agar for further biochemical identification using different API systems.

For *Enterobacteriaceae* and *Pseudomonas aerguinosa* API 20E reagent kit (Biemerieux –France cat# 20-100), for *Staphylococcus* species and CNSAPI-Staph Kit (bioMe`rieux SA, l'Etoile, France), for *streptococcus species* API 20 STREP (bioMe`rieux SA, l'Etoile, France) and for *Candida species* PI 20C AUX (bioMe`rieux SA, l'Etoile, France) were used.

**RESULTS**

One hundred and twelve milk samples from dairy cows were examined clinically for presence of mastitis and by using California mastitis test. They were grouped into; healthy, subclinically and clinically mastitic dairy cows.

According to CMT the prevalence of healthy, subclinical mastitis and clinical mastitis was 30.3, 56.3 and 13.3% respectively as shown in Table (1).

Seventy eight milk samples positive with CMT were examined by SCC and bacteriological examination.

The 63 subclinical mastitis were examined by SCC and the given numbers were 8, 25 and 30 in (< 10³), (>10³ – 3x10⁵) and (>3x10⁵ – 5x10⁵) ranges respectively. On the
Table 1: Prevalence of mastitis by using CMT and SCC in the examined dairy cow milk samples

<table>
<thead>
<tr>
<th>Milk samples</th>
<th>CMT</th>
<th>SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>34</td>
<td>30.3</td>
</tr>
<tr>
<td>Subclinical mastitis</td>
<td>63</td>
<td>56.3</td>
</tr>
<tr>
<td>Clinical mastitis</td>
<td>15</td>
<td>13.3</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Results of bacteriological examination of the positive CMT dairy cow milk samples

<table>
<thead>
<tr>
<th>Bacteriologically positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of bacteriologically examined milk samples</td>
</tr>
<tr>
<td>No. %</td>
</tr>
<tr>
<td>78</td>
</tr>
</tbody>
</table>

Table 3: Biochemical identification of isolated causative agents using API system


Table 4: incidence of bacterial agents from the total examined CMT positive samples

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. of milk samples</th>
<th>No. of isolates</th>
<th>%</th>
<th>Bacterial species</th>
<th>No. of milk samples</th>
<th>No. of isolate</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>67</td>
<td>12</td>
<td>25.5%</td>
<td>S. aureus &amp; E. coli</td>
<td>26</td>
<td>5</td>
<td>17.8%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>7</td>
<td>7</td>
<td>14.8%</td>
<td>S. aureus, S. agalactiae &amp; E. coli</td>
<td>2</td>
<td>2</td>
<td>7.1%</td>
</tr>
<tr>
<td>Coagulase negative Staphylococci (CNS)</td>
<td>6</td>
<td>6</td>
<td>12.7%</td>
<td>S. aureus, E. coli &amp; K. pneumoniae</td>
<td>2</td>
<td>2</td>
<td>7.1%</td>
</tr>
<tr>
<td>St. agalactiae</td>
<td>6</td>
<td>6</td>
<td>12.7%</td>
<td>E. coli &amp; K. pneumoniae</td>
<td>4</td>
<td>4</td>
<td>14.2%</td>
</tr>
<tr>
<td>St. pyogens</td>
<td>5</td>
<td>5</td>
<td>10.6%</td>
<td>S. aureus &amp; K. pneumoniae</td>
<td>4</td>
<td>4</td>
<td>14.2%</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>4</td>
<td>4</td>
<td>8.5%</td>
<td>CNS &amp; E. coli</td>
<td>4</td>
<td>4</td>
<td>14.2%</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>2</td>
<td>2</td>
<td>4.2%</td>
<td>St. agalactiae &amp; E. coli</td>
<td>2</td>
<td>2</td>
<td>7.1%</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>2</td>
<td>2</td>
<td>4.2%</td>
<td>S. pyogens &amp; E. coli</td>
<td>2</td>
<td>2</td>
<td>7.1%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
<td>2</td>
<td>4.2%</td>
<td>CNS &amp; K. pneumoniae</td>
<td>2</td>
<td>2</td>
<td>7.1%</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1</td>
<td>1</td>
<td>2.1%</td>
<td>Proteus spp &amp; S. aureus</td>
<td>1</td>
<td>1</td>
<td>3.5%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>47</strong></td>
<td><strong>total</strong></td>
<td><strong>28</strong></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

In the present study, a total number of 112 milk samples was examined by using CMT for determination of mastitis prevalence in the selected dairy farms. The prevalence of subclinical Mastitis and clinical mastitis were 56.3 and 13.3% respectively as shown in Table (1).

These findings are nearly similar to that recorded by EL-Rashidy et al., [15] who reported that the prevalence of subclinical mastitis was 62.08% amongst the microbiologically examined Friesian cows. Awad [16] recorded that, the prevalence of subclinical mastitis was 69.4%. Meanwhile higher prevalence was recorded by Karimuribo [17] (75.9%), and Kivaria et al. [18] (78%) between the dairy cow farm.

Also finding in the present work are the same as [21] who reported that there is clear correlation between infection and number of SCC.

Regarding the prevalence of clinical mastitis, findings of this study revealed that the percentage of cases was 13.3%.

These results nearly agree with Ahmed [22] who stated that the percentage of clinical mastitis was 9.66%.. Also, Asfour [23] reported that the percentage of clinical mastitis was 3.6% and Petrovski et al. [24] who reported that the percentage of clinical mastitis was 10% in dairy cows. On the other hand, our findings are not supported by those of Tarek [25]. Lakew et al. [20] who reported that the clinical mastitis prevalence was 15%.

Concerning the bacteriological examination of the suspected isolates, the present study revealed that 96% was positive cases mean while Lakew et al. and
Seleim et al., [20, 26] reported that the bacteriological positive samples were 93 and 98 respectively. Also Char et al. [27] reported that bacteriologically positive samples were 86.7% and the bacteriologically negative samples were 13.3%. On the other hand Malinowski et al., [28] found that the bacteriologically negative samples were 32.7%. In this study, the prevalence of different bacteria isolated from the quarter milk samples and related to single infection were recorded, as E. coli (25.5%) representing the highest prevalence rate. Vaarst and Enevaldsen [29] reported that the prevalence of E. coli was 36%. Also, Akram et al., [14] found that E. coli was the common pathogen which can be isolated from the milk samples as an environmental pathogen. Concerning S. aureus and Ps. aeruginosa, they were isolated at a rate of 14.8 and 4.2% which is correlated with EL-Rashidy et al., Almaw and Gizat and Sampimon et al., [16, 30, 31] findings that S. aureus prevalence was (17.03%) and P. aeruoginosa (3.66%). Meanwhile Tufani et al., [32] found that prevalence of Staphylococcus spp. was (66.67%) and E. coli (15.87%).

REFERENCES

15. EL-Rashidy, A.A., A. Amin, A.S. Mostafa and M.S. Tawfik, 1998. Evaluation of mastitis screening test in diagnosis of subclinical cases compared with the conventional bacteriological methods.