ISOLATION AND IDENTIFICATION OF *Staphylococcus aureus* FROM BUFFALOES MILK INFECTED WITH SUBCLINICAL MASTITIS AND MILK WORKERS

Douaa A. Khaleel Rasha M. Othman Bassam Y. Khudaier  
Department of microbiology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.  
(Received 10 November 2015, Accepted 9 December 2015)

**Keywords:** Mannitol salt agar, Buffaloes, *S. aureus*.

**ABSTRACT**

A total of 100/270 (37%) fermented Mannitol salt agar (MSA) isolates were obtained: 40/180 (22.2%) were from buffaloes milk with subclinical mastitis and 60/90 (66.7%) were from hands of milk workers. All suspected *Staphylococcus aureus* isolates which tested microscopically and biochemically were 15/100 (15%) of suspected isolates, 5/40 (12.5) from milk and 10/60 (16.7) from hands swabs of milk workers were diagnosed as *S. aureus*.

**INTRODUCTION**

*Staphylococcus aureus* is one of the most important pathogens in humans and animals [1]. It is an important food-borne pathogen involved in a variety of invasive diseases [2]. There are many diseases affecting the milk yield of buffaloes. Of these, mastitis is at the top[3]. Mastitis (inflammation of mammary gland) is one of the most devastating disease conditions leading to significant economic losses globally [4,5]. Mastitis is the most common infectious disease affecting the dairy buffaloes and remains the most economically important disease of dairy industries around the world. A wide variety of bacteria can be involved, but the most common mastitis pathogens are *S. aureus* [6]. Mastitis, which may be clinical (severe) or subclinical (moderate), is an important mammary gland disease that is usually caused by bacterial infection[7]. *S. aureus* is frequently associated with subclinical mastitis and may contaminate milk and other dairy products [8]. It is usually colonizes in the teat canal initially. After colonization, the bacteria adhere to the epithelium of ducts and alveoli in the gland and starts toxin production. The adherence of bacteria then stimulate
macrophage and migration of neutrophils from blood into the milk which will lead to high somatic cell number (SCC), swelling of the mammary gland, damage in the host defense system and epithelial cells [9].

*S. aureus* is able to produce a host of structural changes in udder and keeps on developing resistance against the most commonly used antibiotics .These resistant bacteria become part of the environment and are transmitted from animals to humans[10].*S. aureus* evolves resistance to many classes of antibiotics [11].The emergence of antibiotic-resistance *S. aureus* strains resulted in significant treatment difficulties which imposed burden on health care systems and simultaneously intensifying the need for new antibiotics [12].

The present study was conducted to isolation and identification *S. aureus* from buffaloes with subclinical mastitis and milk workers.

**MATERIAL AND METHOD**

**Collection of Samples**

One hundred and eighty milk samples were collected from buffaloes from different regions in Al-Basrah province .Prior to sampling, the California Mastitis Test (CMT) was carried out. Milk samples were collected from buffaloes milk after cleaning the udder from the grimes, bole and dirt by water and drying by a piece of clean cloth then used cotton moistened by alcohol 70% and removing the first flowage of milk and collecting 10 ml in sterile tube, transported by ice box immediately to the laboratory. Ninety samples were collected from humans hands swabs (milk workers), then direct transported to the laboratory.

**Identification of *S.aureus***

Identification of *S.aureus* was carried out according to [13], each sample from milk and hand swab samples were directly inoculated onto mannitol salt agar (MSA) and incubated at 37 ℃ for 24 hrs .Mannitol fermented colony from primary cultures were purified by subculture onto MSA medium and incubated at 37 ℃ for 24-48 h. Gram stain slides were investigated according to [14] and biochemical tests that included catalase and coagulase tube were performed according to [15].
Biochemical Tests

Tube Coagulase Test

A 0.1 ml of 18-24 h bacterial cultured in brain heart infusion broth was added to 0.3 ml of rabbit plasma without dilution and incubated at 37°C for 4 h. The clotting hourly noticed, then tubes was left at room temperature for 18-24 hrs. The appearance of the clotting indicates as a positive result comparable to control [15].

Catalase Test

The catalase test was carried out according to [15] as following: A small amount of pure growth was transferred with a wooden stick from MSA into clean slide, then a drop of catalase reagent was added. The evolution of gas bubbles indicates a positive result.

RESULTS

Table (1) shows the results of bacterial culturing on MSA 100 (37.03%) isolates out of 270 tested samples were suspected S. aureus. The percentage of frequency of S. aureus isolates were 40/180 (22.2%) and 60/90 (66.6%) for buffaloes milk samples and humans hand swab samples, respectively. On the other hand the biochemical tests of S. aureus isolates revealed that the number and percentage of S. aureus isolates were 5/40 (12.5%) for buffaloes milk and 10/60 (16.6%) in humans hands swabs, Table (2).

Table (1): Prevalence of S. aureus Isolated from Buffaloes Milk and Hands Swabs From Milk Workers.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>No. of samples</th>
<th>No of S. aureus fermented MSA</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk from buffaloes</td>
<td>180</td>
<td>40</td>
<td>22.2</td>
</tr>
<tr>
<td>Hands swabs</td>
<td>90</td>
<td>60</td>
<td>66.6</td>
</tr>
<tr>
<td>Total</td>
<td>270</td>
<td>100</td>
<td>37.03</td>
</tr>
</tbody>
</table>

306
Table (2): Number and Percentage of S aureus with Biochemical Test.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>S. aureus fermented MSA</th>
<th>Coagulase test (%)</th>
<th>Catalase test (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bufaloes milk</td>
<td>40</td>
<td>5 (12.5 %)</td>
<td>40 (100 %)</td>
</tr>
<tr>
<td>Hands swabs</td>
<td>60</td>
<td>10 (16.6 %)</td>
<td>60 (100 %)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>15 (15 %)</td>
<td>100 (100 %)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

*S. aureus* is important milk borne pathogen and causes a wide variety of humans and animals diseases and it is frequently associated with subclinical mastitis in dairy animals and may contaminate milk and other dairy products which act as vehicles for *S. aureus* infection in humans[16].

*S. aureus* can be transmitted to humans through contaminated and untreated milk and milk products[17]. *S. aureus* presents on the skin and mucosa of food producing animal reservoirs that include ruminants and it is frequently associated with subclinical or clinical mastitis leading to the contamination of dairy products[18].

In present study (12.5 %) *S. aureus* strains were isolates from subclinical buffaloes mastitis that diagnosed by culturing, microscopically examination and biochemical tests, this percentage of *S.aureus*infection was similar to result that recorded by,[19 ,20,21] which were 10.23%, 10.9% and 15.62%, respectively. While the highest incidence of buffaloes subclinical mastitis were 78.12%; 58.33%; 48%;34.6%and 25.53%as reported (22, 23,24,25,26) respectively. The frequency of *S. aureus* isolated in the present study was 10 (16.66%) out of 60 hand swabs of dealers, these result is nearly to the result was reported by.[27].

In the other hand,[24] and,[28] were found more highest rates of *S. aureus* isolates from hand swabs of milk workers 70% and 56.52% ,respectively, in comparison with the rate of the present study, while [29] were found that 40% of *S.
aureus in animal workers. Therefore, a comparison of the results of the present study and those reported by other authors is difficult because the occurrence of *S. aureus* as a causative agent of mastitis varies according to the area, handling practices of the animals and hygienic conditions during milking [30]. To reduce the risk of the presence of *S. aureus* and other microorganisms in raw milk, it is necessary to gadget measures to reduce the prevalence of intramammary infections as well as increase the development of guidelines and support for dairy producers to improve production techniques that enhance the quality of milk in terms of microbiological, physical-chemical, sensual and nutritional aspects [31].

REFERENCES


19-Khudaier, B. Y.; Anad, I. T. and Abbas, B. A.(2014). Isolation of *Staphylococcus aureus* from Buffalo Milk in Basra Governorate and Detection of Their...


