THE PREVALENCE AND SOURCE OF STAPHYLOCOCCUS AUREUS IN MILK IN DIYALA PROVINCE

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ABSTRACT
The presence of Staphylococcus aureus in the raw milk leading to serious public health hazards and their counts are used as an index of the proper sanitation quality of the dairy products. A total of 20 raw milk samples were obtained from the individual clinical and subclinical mastitic cow’s udders (10 samples for each) and other 20 raw milk samples were obtained from the 50 kg milk cans and the 5 tons bulk milk tanks (10 samples for each) from the different rural areas of the Diyala province. All the milk samples was collected at weekly intervals during the period that extended from the January to the end of March 2017. Raw milk sample was transported to the laboratory inside ice cooled box for the microbiological analysis to isolate, identify and enumerate the Staph. aureus in all the samples. The laboratory studies of the cultural isolation in the current study found that the a significant (p<0.05) high prevalence levels of Staph. aureus in the milk samples of both the clinical and subclinical mastitic cow’s udders (100% & 70 % respectively). The prevalence increased significantly (p<0.05) to (50%) in the milk samples of the bulk milk tanks, The current microbiological studies detected the statistically significant (p<0.05 ) influence of the mastitis on the total viable Staph. aureus counts in the raw milk which was found that all the milk samples that were obtained from the clinical and subclinical mastitic udders had significantly (p<0.05) higher mean log values of total Staph. aureus counts by using the chromogenic agar (6.65 and 6.42 log cfu / ml respectively) than those that were obtained from the milk cans and bulk milk tanks (6.29 and 6.30 log cfu / ml respectively).

KEYWORDS: health hazards, microbiological analysis, mastitis udders, chromogenic agar.

INTRODUCTION
Milk understandably an important constituent of human diet and raw milk is a perfect development medium for a few microorganisms. Milk and its derivate are considered vehicles for Staphylococcus aureus disease in people (Zecconi and Hahn, 2000). In dairy cows Staph. aureus is much of the time related with subclinical mastitis and may debase milk and other dairy products (Jones et al., 2006). Although pasteurization is likely to destroy all pathogens, there is concern when raw milk is consumed or when pasteurization is incomplete or faulty Staph. aureus produces a few staphyloccoca destructiveness factors, including enterotoxins (SEA to SEE and SEG to SEQ), and different toxins, for example exfoliative toxin A and B, and Toxic Shock Syndrome Toxin (TSST-1) (Fagundes and Oliveira, 2004) and milk of the infected animal is the main source of enterotoxigenic Staph. aureus of animal origin and these toxins are known to cause nausea, vomiting and abdominal cramps when ingested by human and are responsible for staphylococcal food poisoning outbreak (Loncarevic et al., 2004 and Kerouanton et al., 2007). Staphylococcus aureus in raw milk by and large originates from dairy animals with mastitis, from handlers or from inadequate cleanliness.

MATERIALS & METHODS
A total of twenty raw milk samples were obtained from the individual cows that infected with either clinical mastitis or subclinical mastitis (10 samples for each) located inside the farmer’s homes distributed in different rural areas of Diyala province. In addition to that, other twenty raw milk samples were obtained from both the 50Kg capacity milk cans and the 5 tons capacity bulk milk tanks (10 samples for each) from different rural areas inside the Diyala province. All raw milk samples were collected randomly at weekly intervals in a sterile polyethylene plastic bags (500 ml capacity) during the period that extended from January to the end of March 2017. All the raw milk samples were kept inside ice-cooled box and transported immediately within 2 hours to the milk laboratory at the department of veterinary public health, college of veterinary medicine, university of Baghdad. The microbiological analysis was performed on the arrival of the milk samples to isolate, identify and enumerate the Staphilococcus aureus in the milk samples. dilutions (10^1 to10^7) for each milk sample tenfold decimal serial were prepared in a sterile 0.1 % (wt/v) buffered peptone water as a diluent and pour plated (APHA 2001). Plating was done within 2 hours of the arrival of the samples to the laboratory. Tenfold decimal serial dilutions were prepared by using a suitable diluent and then pour plated in duplicate for each dilution Staphylococcus.
Staphylococcus aureus colonies were enumerated after aerobic incubation at 37°C for 48 hours. Plates that had 25-250 colonies were selected for counting using the colony counter with magnifying lens. The total average number of Staph. aureus colonies multiplied by the appropriate dilution factor to get the Staph. aureus counts per milliliter of milk or nutrient broth (cfu / ml). The Staph. aureus cultures were isolated from milk, sample after 48 hours of aerobic incubation at 37°C on the selective chromogenic agar. The representative Staph. aureus colonies were randomly picked up from the chromogenic agar and then purified by two successive streaking on the chromogenic agar. Staph. aureus isolates were identify on the bases of cultural, morphological, biochemical and serological characteristic (Ogden et al., 2001). Staph. aureus isolates were identified macroscopically with the respect of cultural characteristics such as the surface, color, shape and size and microscopically with the respect of cells shape and arrangement. The Staph. aureus slide was examined under the high power magnification to investigate the morphological features of the cells.

**TABLE 1.** The prevalence of *Staphylococcus aureus* in both the mastitic and fresh raw milk sample collected from Diyala province by using the conventional cultural methods

<table>
<thead>
<tr>
<th>Type of milk samples</th>
<th>Number of examined samples</th>
<th>Number of positive samples</th>
<th>Isolation Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical mastitic Milk</td>
<td>10</td>
<td>10</td>
<td>100a</td>
</tr>
<tr>
<td>Sub clinical mastitic Milk</td>
<td>10</td>
<td>7</td>
<td>70ab</td>
</tr>
<tr>
<td>Milk cans (50kg)</td>
<td>10</td>
<td>3</td>
<td>30c</td>
</tr>
<tr>
<td>Bulk milk tank (5tons)</td>
<td>10</td>
<td>5</td>
<td>50bc</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>25</td>
<td>0.39</td>
</tr>
</tbody>
</table>

* Different capital letters in the column revealed significant (p<0.05) differences between milk samples types.

The unhygienic practices and poor sanitation techniques in the milking process with improper handling, storage and distribution may introduced such organism in the milk and reflected on the high prevalence level of contamination with such organism which was responsible for many outbreaks of food poisoning by the consumption of the raw dairy products (Veras et al., 2008). The current results disagreed with Peles et al. (2007) who indicated that lower prevalence rates of *Staph. aureus* was found in the bovine raw milk samples.

The present result was disagreed with AL-Idani (2016) who showed that the percentage of *Staph. aureus* isolated from the positive California mastitis test was 36.7%. The results of the present study were in agreement with Petersson-wolf et al. (2010) and AKineden et al., (2011) who recorded that the higher prevalence rate of *Staph. aureus* occurred by shedding of this bacteria because the *Staph. aureus* was the major causative agent of the subclinical mastitis in the dairy cows. Investigations on other countries, Farhan salkj (2007) revealed that 48 out of 130 (36.9%) cow’s raw milk samples in Palestine were containing *Staph. aureus* Ekici et al., (2004) found that (18.18%) of 66 cow’s raw milk samples in Turkey were positive for *Staph. aureus*.

**TABLE 2:** The mean values of *Staphylococcus aureus* counts (log cfu/ml) in both the mastitic and fresh raw milk samples using the mannitol salt agar

<table>
<thead>
<tr>
<th>Type of milk samples</th>
<th>Number of examined samples</th>
<th>Counts of <em>Staph. aureus</em> Log cfu/ml Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk cans (50kg)</td>
<td>10</td>
<td>6.19±0.04 c</td>
</tr>
<tr>
<td>Bulk milk tank (5tons)</td>
<td>10</td>
<td>6.25±0.02 c</td>
</tr>
<tr>
<td>Individual clinical mastitic Milk</td>
<td>10</td>
<td>6.61±0.02 a</td>
</tr>
<tr>
<td>Individual sub clinical mastitic milk</td>
<td>10</td>
<td>6.38±0.04 b</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>6.35±0.04 b</td>
</tr>
<tr>
<td>LSD</td>
<td>0.0983</td>
<td></td>
</tr>
</tbody>
</table>

* Different capital letters in a column revealed significant (p<0.05) differences between types of milk samples. SE = standard error

The microbial populations (counts) were proved to be efficient as an indicator of good or poor sanitary conditions in milk production. The results of the current study showed Table (1.2) established that both milk...
samples collected from the milk cans and tanks had significantly (p<0.05) the lowest Staph. aureus counts (6.19 & 6.25 log cfu/ml respectively) in comparison to those samples that obtained from both the clinical and subclinical mastitic udders (6.6 and 6.38 log cfu / ml respectively). An overall conclusion on the bases of the present investigation pointed out the highest contamination levels by shedding the viable Staph. aureus in milk were found in both the clinical and subclinical mastitic milk samples Pelisser et al. (2009) reported that Staph. aureus counts should reach approximately 10^7 to 10^9 cfu /gm or ml (4-5 log cfu / gm or ml ) to produce the enterotoxins and cause food poisoning. Mastitis is caused by a wide spectrum of pathogenic microorganisms that penetrate the teat canal and multiply in the udder cistern, but the majority of the mastitic cases were produced by the Staph. aureus ( Bramley and Dodd, 1984 ). Microbial contamination occurred mainly during and after the milking process where microorganisms were introduced to the milk by a number of ways, such as excretion from the interior of infected udders, or contamination from the dairy far environment (Vissers and Driehuis, 2009). The presence of Staph. aureus in the raw milk was an indication of unhygienic practices during the milking process and/or improper handling of the milk (EL-Zubeir and Ahmed, 2007).

**REFERENCES**


**Staphylococcus aureus in milk in Diyala Province**


