INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

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 (6.65and 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 derivates 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 staphylococca 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 *Staphylococcus 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*

aureus colonies were enumerated after aerobic incubation at 37ºC 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 37ºC 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.

RESULTS & DISCUSSION

A total of forty raw milk samples were collected from the farmers homes that distributed in different region of Diyala province where 20 samples of them were collected from the mastitic udder´s and the other 20 samples from the 50 kg milk cans and the 5 tons bulk milk tanks. The data shown in Table 1.1 showed that 25 out of 40 (62.5%) fresh and mastitic milk samples were positive for the presence of *Staph. aureus* and such high prevalence value revealed that the cows producing milk in the rural areas of Diyala province need an attention for cleaning management and the udders should be routinely checked for somatic cell counts every six months or using the California mastitic test to identify the infected udders by the *Staph. aureus*. The highest significant ($p<0.05$) isolation percentage was recorded in the clinical and subclinical mastitic milk samples (100% &70% respectively) whereas both samples that were collected from milk cans and milk tanks showed lower isolation percentage (30% and 50% respectively).

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

Type of milk Samples	Number of Examined samples	Number of positive Samples	Isolation Percentage
Clinical mastitic Milk	10	10	100a
Sub clinical mastitic Milk	10	7	70ab
Milk cans (50kg)	10	3	30c
Bulk milk tank (5tons)	10	5	50bc
Total	40	25	0.39

* 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

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

* 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 10^4 to 10^5 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 diary 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).

TABLE 3: Comparison of *Staphylococcus aureus counts* (log cfu /ml) in both the mastitic and the fresh raw milk samples between the mannitol salt agar and the chromagar

Type of milk samples	Number of examined samples per agar	*Staph. aureus counts(Log cfu/mL)* Mean ± SE	
		Mannitol agar	Chrom agar
Milk cans (50kg)	10	6.19 ±0.04 aC	6.29±0.06 aC
Bulk milk tank (5tons)	10	6.25 ±0.02 aC	6.31 ±0.02 aC
Individual clinical mastitic Milk	10	6.61 ±0.02 aA	6.65 ±0.02 aA
Individual sub clinical mastitic milk	10	6.38 ±0.04 aB	6.42 ±0.02 aB
Total	40	6.35 ±0.04	6.42 ±0.03
LSD		0.1789	

* Means with different capital letters in the same column significantly different ($p<0.05$)
*Means with different small letters in the same row significantly different ($P<0.05$)
* SE= standard error

The results illustrated in Table 1.3 showed that the chromogenic and the mannitol salt pour plating methods were similar in the sensitivity for counting the *Staph. aureus* in the milk samples and the difference between both methods was less than 0.1 log cfu /ml for all the four types of milk samples .

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