Global Veterinaria 11 (4): 403-405, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.11.4.76107

Advanced Detection of *Staphylococcus aureus* Enterotoxins in Milk

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Abstract: A total of 120 raw milk samples were collected from different animal farms at Giza governorate. Each sample was divided into two parts; one part was kept at room temperature and the other part was kept at refrigeration temperature. All the parts were subjected for bacteriological examination for isolation and identification of *S. aureus*. 31 out of 120 and 29 out of 120 raw milk samples kept at room and refrigeration temperature, respectively contained *S. aureus*. 26 isolates were underwent reversed passive latex agglutination technique for detection of enterotoxigenic *S. aureus* (11 isolates). No enterotoxigenic *S. aureus* were detected in pasteurized milk incubated at room and refrigeration temperatures.

Key words: *Staphylococcus Aureus* · Enterotoxins · Milk · Reversed Passive Latex Agglutination Technique

INTRODUCTION

Milk is considered a good medium for the growth of microorganisms including *Staphylococcus aureus* (*S. aureus*). S. aureus is a facultative anaerobic Gram-positive coccus; it is non-motile, catalase and coagulase positive. Cells are spherical single or paired or form grape-like clusters, the staphylococcal cell wall is resistant to lysozyme and sensitive to lysostaphin [1].

S. aureus is the most common cause of gastroenteritis resulting from the consumption of contaminated food in which enterotoxigenic staphylococci have grown and produced toxins. As these toxins are excreted from the organism, they are referred to as exotoxins; however, they normally exert their effects on the gastrointestinal tract and therefore are called enterotoxins. Staphylococcal enterotoxins are considered a potential biological threat because of their stability at 100°C for 1 hour [2].

The staphylococcal enterotoxins are short proteins secreted in the medium and soluble in water and saline solutions. They are highly stable, resist most proteolytic enzymes such as pepsin, trypsin or chymotrypsine and thus keep their activity in the digestive tract after ingestion and absorption resulting in staphylococcal food poisoning [1].

There is a broad range of classic antigenic staphylococcal enterotoxins (SE) including to date, 23 different SEs have been described: they are designated SE A to X and have been sequenced and described [3-6].

The detection of (SE) is of public health significance and is epidemiologically essential. As the milk is the most important food in our country and the risk of poisoning from the ingestion of milk containing enterotoxin is well known. This study was planned to detect enterotoxins produced by *S. aureus* isolates using reversed passive latex agglutination technique (RPLA) and determine their production using cultured milk at room and refrigeration temperatures.

MATERIALS AND METHODS

A total of 120 random raw milk samples were collected from different animal farms at Giza governorate, Egypt. Each sample was divided into two parts; one part was kept at room temperature and the other part was kept at refrigeration temperature.

Ten-fold serial dilutions were prepared and from each dilution 1 ml was aseptically transferred and inoculated onto 3 plates of Baird-Parker medium (Lab M), the plates were incubated for 24-48 hours at 37°C. The plates containing 20-200 colonies were selected. Typical

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colonies of *S. aureus* were circular, smooth, convex, moist, 2-3 mm in diameter, gray to black (potassium tellurite reaction) with white margin and surrounded by outer clear zone (egg yolk reaction).

Suspected colonies were streaked onto agar slant of nutrient medium [7] and incubated at 37°C for 24 hours for further identification by microscopical examination, catalase, coagulase, thermostable nuclease and Voges-Proskauer tests.

S. aureus culture supernatant were collected by Sac cultural method [8] and tested serologically by reversed passive latex agglutination technique using Oxoid SET-RPLA kits for the presence of SEA, B, C and D).

Detection of enterotoxins production by *S. aureus* isolates in cultured pasteurized milk at room and refrigeration temperatures was done as follows:

- Each *S. aureus* isolate was inoculated into BHI broth for 24 hours at 37°C. Each laboratory pasteurized milk sample was divided into four parts; two parts were cultured for enterotoxigenic *S. aureus* at a rate of 10^8 /ml while the other two parts were left as control (NHS, 2005). One cultured and one control milk samples were kept at room temperature and the other two at the refrigeration temperature. The milk samples were tested serologically by SET-RPLA.
- Extraction of *S. aureus* enterotoxins from milk samples were completed by blending of 10 ml of milk sample with 10 ml of sodium chloride solution (0.85%) and centrifuged. The supernatant was retained for toxin detection using Oxoid SET-RPLA kits [9].

RESULTS

31out of 120 staphylococci isolated from raw milk kept at room temperature, proved to be *S. aureus* while 29 out of 116 staphylococci isolated from raw milk kept at refrigeration temperature proved to be *S. aureus*.

It was evident that 11 out of 26 (42.3%) *S. aureus* isolates proved to be enterotoxigenic. SEA was the most frequent detected either alone or in combination with other types C and D.

S. aureus count in cultured pasteurized milk (initial inoculums was $8x10^8$ c. f. u. /ml) kept at room temperature for one day, increased gradually till reach a range of $8.7x10^{12}$ - $4.7x10^{15}$ c. f. u. /ml. Enterotoxins couldn't be detected even after 24 hours of incubation at room temperature.

Table 1: Correlation of *S. aureus* count and enterotoxin production in cultured pasteurized milk kept at refrigeration temperature

Serial No. of isolate	Incubation time	Count	Enterotoxin production
Isolate 1	24 h	$3.2x10^{10}$	-ve
	48 h	$1.2x10^{13}$	-ve
Isolate 2	24 h	$7.3x10^{9}$	-ve
	48 h	$4.8x10^{12}$	-ve
Isolate 3	24 h	$4.7x10^{10}$	-ve
	48 h	$8.6x10^{13}$	-ve
Isolate 4	24 h	$5.3x10^{10}$	-ve
	48 h	$1.3x10^{13}$	-ve
Isolate 5	24 h	$2.5x10^{11}$	-ve
	48 h	$1.8x10^{14}$	-ve
Isolate 6	24 h	$4.1x10^{11}$	-ve
	48 h	$1.9x10^{13}$	-ve
Isolate 7	24 h	$8.3x10^{10}$	-ve
	48 h	$1.6x10^{13}$	-ve
Isolate 8	24 h	$7.5x10^{11}$	-ve
	48 h	$9.5x10^{13}$	-ve
Isolate 9	24 h	$4.4x10^{10}$	-ve
	48 h	$8.7x10^{12}$	-ve
Isolate 10	24 h	$3.7x10^{11}$	-ve
	48 h	$1.4x10^{14}$	-ve
Isolate 11	24 h	$1.0x10^{11}$	-ve
	48 h	$3.1x10^{13}$	-ve

S. aureus count in cultured pasteurized milk (Initial inoculums was $8x10^8$ c. f. u. /ml) kept at refrigerator for 2 days, increased gradually till reach a range of $4.8x10^{12}$ – $1.8x10^{14}$ c. f. u. /ml. Enterotoxins couldn't be detected even after 48 hours of incubation at refrigeration temperature as showed in Table (1).

DISCUSSION

Staphylococcal food poisoning is of major concern in public health programs worldwide. Predictive models for *S. aureus* growth and SEs production would be powerful tools for microbial risk assessment in food industries. However, many factors affect *S. aureus* growth and SE production in milk [1].

Staphylococci count of the raw milk kept at the room temperature was higher than that of the raw milk kept at the refrigeration temperature for the same time.

There was a good correlation between coagulase and thermo-stable nuclease reaction. Isolates showed 3+ or 4+ positive coagulase reactions also produced thermo-stable nuclease and this agreed with Garcia [10] and Rea [11].

Voges Proskauer test was used to detect acetoin production and was the key test to differentiate *S. aureus* from other coagulase positive staphylococci.

S. aureus was one of the dominant bacteria associated with raw milk samples. The presence of these bacteria in milk suggested contamination from various sources such as animal, human, environment, utensils and others. The high numbers of the microorganisms not only contaminate the milk but also multiply and grow in it. This might be due to the fact that milk is a good nutritive medium for the growth of microorganisms especially with poor sanitary procedures and lack of the cooling facilities [12].

The essential goal of the present study was to determine the effect of different temperatures (room and refrigeration) on the production of enterotoxins by *S. aureus* in milk.

S. aureus count in cultured pasteurized milk (Initial inoculum was 8×10^8 c. f. u. /ml) kept at room temperature for 24 hours, increased gradually till reached a range of $8.7\times10^{12} - 4.7\times10^{15}$ c. f. u. /ml. Enterotoxins couldn't be detected even after 24 hours of incubation at room temperature while the data in Table (1) reported that *S. aureus* count in cultured pasteurized milk (Initial inoculums was 8×10^8 c. f. u. /ml) kept at refrigerator for 48 hours, increased gradually till reach a range of $4.8\times10^{12} - 1.8\times10^{14}$ c. f. u. /ml. Enterotoxins couldn't be detected even after 48 hours of incubation at refrigeration temperature.

Enterotoxins couldn't be detected in cultured milk with enterotoxigenic *S. aureus* kept at room and refrigeration temperatures in spite of good growth correspondence due to subculture of the organism in addition to unfavorable incubation temperatures.

CONCLUSION

SE A was the most frequent detected either alone or with other types C or D.

It is concluded that simultaneous efficient and rapid detection of staphylococcal enterotoxins coupled with the specific detection of the producing species indicates the potential to have the hazardous dairy food products.

The rapid and efficient detection of SE in raw milk and finished dairy products by RPLA is essential for consumer safety. This study shows that raw milk can be tested for SE with rapid simple RPLA procedures.

Raw milk was heavily contaminated by *S. aureus* (including enterotoxigenic strains) which is in partly due to growth medium properties of milk.

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