# Enterotoxogenic *staphylococcus aureus* isolated from soft cheese.

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## A B S T R A C T

**A** total of 45 random samples of locally white soft cheese were collected from various dairy shops, street vendors and supermarkets located in Gharbia governorate, Egypt. The samples were represented as Domiati, Tallaga, and Kareish cheese (15 samples of each) were examined for the presence of *Staphylococcus aureus* organisms. The incidence of Staph. *aureus* were 13.3%, 26.6% and 26.6% in the examined cheese samples respectively, with a mean counts/ml of $6.2\times10^2 \pm 2.6\times10^2$ , $7.1\times10^2 \pm 2.8\times10^2$ and $1.2\times10^2 \pm 0.4\times10^2$ respectively. Out of 10 isolates of *Staph. aureus* , only 2 strains (20%) , (1each from) Tallaga and Kareish cheese were enterotoxigenic belonging to enterotoxin A and 2 strains (20%) belonging to A, C enterotoxin while the corresponding cheese samples were enterotoxins free

**Keywords:** *Staph. aureus*, Enterotoxins, cheese.

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## 1. INTRODUCTION

Cheese is universally recognized as first class food. Production and handling of locally manufactured Egyptian white soft cheeses, is still under-way especially those produced by cheese maker in most villages distributed all-over the country. Therefore, such cheese is mostly contaminated with different types of organisms gaining access to the product from various sources. *Staphylococcus aureus* is a food born pathogen responsible for an intoxication resulting from the ingestion of food containing preformed heat-stable enterotoxins, usually produced by this microorganism and representing a sanitary risk when levels of specific bacterial counts at least as high as $10^5$ cfu/ g or ml of sample are detected (Jablonski and Bohach, 1997**).** The presence of *Staphylococcus aureus* in cheese constitutes a potential public health hazard since many strains of *Staphylococcus aureus* produce enterotoxins that cause food poisoning if ingested. Neither the absence of *Staphylococcus aureus* nor the presence of small numbers is complete assurance that a food is safe. Conditions inimical to the survival of *Staphylococcus aureus* may result in diminishing operation or death of viable microbial cells, while sufficient toxins remain to elicit symptoms of staphylococcal food poisoning. The most common symptoms are nausea, vomiting and diarrhea. However, in severe cases they may be accompanied by acute prostration and abdominal cramps. Symptoms usually occurring 2 to 6 hrs after ingestion of the contaminated food (AOAC, 1984 and Lancette and Tatini, 1992).

So this work was undertaken to detect the presence of enterotoxogenic strains of *Staphylococcus aureus* in locally purchased soft cheese samples and to determine whether this examined cheese samples is a potential vehicle for such enterotoxins or not.

## 2. MATERIALS AND METHODS

### *2.1. Collection of samples*

A total of 45 random samples of soft cheese (15 each of) Domiati, Tallaga and Kariesh

cheese were collected from street paddlers, dairy shops and supermarkets

*2.2. Preparation of samples*

Ten grams from each sample were homogenized with 90 ml sterile 0.2 % sodium citrate solution in a stomacher bag (Lab-blender 400, Seward, UAC House Friars Road, London SE19UG. Model No. 6021). One ml from the original sample homogenate was added to a test tube containing 9 ml 0.1% sterile peptone water to provide a dilution of $10^2$. Similarly a tenfold serial dilution were prepared (APHA, 2001*)*

*2.3. Bacteriological examination*

*a. Staphylococcus aureus presumptive count /ml according to (APHA, 2001).*

From each dilution 0.1ml was spread over a dry surface of Baird parker agar plate (Oxoid CM 275, SR 54). Inoculated plates were incubated at 37°C for 48hours. Typical colonies of *Staph.aureus* (black shining convex colonies, 1-1.5 mm in diameter with narrow white margin and surrounded by a clear area extending into opaque medium) were counted and the average number per gram was calculated.

*b. Isolation and identification of Staphylococcus aureus*

Suspected colonies were picked up onto slants of nutrient agar and incubated at 37°C for 24 hrs. Isolation strains were purified before being subjected for further identification according as the follows: Gram's stain, catalase activity, detection of hemolysis, oxidation- fermentation, coagulase and isolates proved to be staphylococcus were recorded (Quinn, et al., 2002).

*c. Enterotoxins production of isolated strains*

Isolated Staphylococcus aureus strains were examined for their ability to produce enterotoxins using sac culture method (Donnelly et al., 1967).

*d. Detection and typing of enterotoxins:*

According to Oda et al. , (1979) and Shingaki et al.(1981) using serological test by reversed passive Latex agglutination technique using Oxoid SET REPLA (kits used for the detection of *staphylococcal aureus* enterotoxins A, B, C, D and E) .

## 3. RESULTS

Table (1): Incidence of *Staphylococcus aureus* in examined cheese samples

| Samples | Total No. of samples | Positive samples | % |
|---|---|---|---|
| Domiati | 15 | 2 | 13.3 |
| Tallaga | 15 | 4 | 26.6 |
| Kareish | 15 | 4 | 26.6 |

Table (2): Statistical analytical results of *Staphylococcus aureus* count/ml of examined cheese samples.

| Samples | Minimum | Maximum | Mean±SE |
|---|---|---|---|
| Domiati | 24×10 | $10\times10^2$ | $6.2\times10^2\pm2.6\times10^2$ |
| Tallaga | 4×10 | $16\times10^2$ | $7.1\times10^2\pm2.8\times10^2$ |
| Kareish | 4×10 | 25×10 | $1.2\times10^2\pm0.4\times10^2$ |

Table (3): Incidence and distribution of enterotoxigenic *Stapylococcus aureus* isolated from cheese samples.

| No. of +ve Enterotoxigenic strains | | Type of samples | Types of enterotoxin | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E | (A, C) |
| No | % | | | | | | | |
| 2/10 | 20 | Tallaga | 1 | - | - | - | - | |
| 2/10 | 20 | Kariesh | 1 | | | | | 2 |

## 4. DISCUSSION

*Staph. aureus* is an important food born pathogen and a major cause of food poisoning outbreaks worldwide. The presence of *S. aureus* in ready to eat food which are eaten without cooking could be a bacterial risk for humans (Odumeru, et al, 1997). In the present study, Staphylococcus species were detected from the examined white soft cheese samples (Domiati, Tallaga and Kareish ) with an incidence 13.3, 26.6 and 26.6 %, respectively as shown in Table (1). These findings nearly similar with a previous study for incidence of *Staphylococcus aureus(* 28 %) in Domiati soft cheese by El-Sayed, et al, (2011) and lower than these findings achieved by El-Jakee, et al (2013***),*** who isolated *Staph. aureus* in percentage of 4% in soft cheese samples while higher findings achieved by Garbaj et al, (2007) who isolates *Staph.aureus* in 36 and 62 % in soft cheese while Agban and Ahmed (2012*)* detected *Staph. aureus* in 92.8 % in kariesh cheese samples. Staphylococci food poisoning resulting from contaminated milk and dairy products, especially cheeses produced from raw milk in unclean conditions, causes staphylococcal intoxication (Can and Celik, 2012*).* Differences between the results may be based on the differences in the cheese production techniques, storage conditions, type of cheese and whether the milk used was raw or pasteurized. It could be also related to the unclean conditions where the cheese is produced and the personnel involved in production.

In Table (2), the mean levels of *Staphylococcus aureus* counts were $6.2\times10^2\pm2.6\times10^2$, $7.1\times10^2\pm2.8\times10^2$ and $1.2\times10^2\pm0.4\times10^2$ in Domiati, Tallaga and Kareish cheese respectively, which were comparable to those found in such highly contaminated cheese samples ( $1.7\times10^4$ for Domiati , $1.3\times10^5$ for Bramily, $1.2\times10^5$ for Fayomi and $3.2\times10^5$ for Tallaga ) revealed by El-Sayed, et al,(2011) and those reported by Fadel and Ismail (2009) the mean value of Staph. aureus counts was 5.59 $\log_{10}$ cfu $g^{-1}$ . In addition, (Bahout and Moustafa 2006) reported that *Staph. aureus* was present in 28% of the examined Kareish cheese samples with min. , max. , and mean count of $11\times10^2$ , $6.5\times10^5$ and $3.4\times10^4$ cfu $g^{-1}$, respectively, EL-Sayed et al (2011) found higher results for the incidence and counts of these pathogens in Tallaga cheese samples collected from Cairo & Giza areas during 2004 to 2005 .In Domiati cheese, (Nour et al., 1987 &1992 and El Zayat., 1988) and (Kaldes., 1997) reported the presence of *Staph. aureus* in Domiati cheese samples collected from different sites in Egypt with nearly similar incidence close to that obtained in the current study. They also pay similar attention to the probable intoxication due to enterotoxins might be produced at the optimal level of contamination and conditions. Similar counts were obtained by Abou Dawood et al, (2005***)*** for *Staph. aureus* but much higher incidence than that found in the current study.

Results in Table (3) showed that out of 10 isolates of *Staphylococcus aureus* strains, 2 (20%) were toxigenic which belonging to enterotoxin A, one isolated from kareish cheese sample and the other one from Tallaga cheese. Another 2 (20%) enterotoxigenic strains have the ability to produce both A and C enterotoxins were found in 2 Kareish cheese isolates, the same cheese samples were examined for the presence of the typed toxins by SET REPLA according to Oda et al. (1979) and Shingaki et al. (1981) and neither the A nor C enterotoxin were found in the samples and this may be due to absence of optimal level of contamination and storage conditions. The *Staphylococcus aureus* enterotoxins are produced during active growth of the microorganisms in the foods and often during storage. Each enterotoxin is a single polypeptide chain which resists many proteolytic enzymes and withstands boiling for up to 30 minutes (heat stable) although the vegetative cells would not survive such conditions (Eley, 1992).

Finally, there is a great need for rising up, developing and spreading the hygienic knowledge, attention and control measures where cheese is made, handled and served for the public health good

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**المكور العنقودي الذهبي المنتج للسموم المعويه المعزول من الجبن الطري.**

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الجبن الابيض يعتبر من الاغذيه الاساسيه في حياه الانسان التي لا غني عنها في كل بيت وهو من الاغذيه التي تؤكل مباشره بدون التعرض لحراره الطهي لذلك وجب الاهتمام بدراسه الحاله الصحيه له والكشف عن مايحتويه من ميكروبات ضاره مسببه للتسمم الغذائي للانسان ومن اهم هذه الميكروبات، ميكروب المكور العنقودي الذهبي وخاصه العترات التي تفرز السموم المعويه وذلك لخطورتها على صحة المستهلك عن طريق افرازها للسموم المقاومه للحراره المستخدمه في التصنيع والتي تسبب حالات التسمم الغذائي للإنسان. لذا اجريت هذه الدراسه للكشف عن مدي تواجد تلك العترات من الميكروب العنقودي في 3 انواع من الجبن الابيض الشائع الاستهلاك بين طبقات المجتمع المختلفه وهم الجبن الدمياطي وجبن الثلاجة والجبن القريش. تم تجميع 45 عينه (15 من كل نوع) من محلات الالبان والسوبر ماركت والباعه الجائلين في الاسواق المحليه بمحافظه الغربيه ، وقد اظهرت النتائج تواجد ميكروب المكور الذهبي في العينات المختبره بالنسب الاتيه 13,3 ، 26,6 و 26,6 % في العينات علي التوالي وبمتوسط عدد كلي للميكروب كالاتي : $10^2$ ×2,6±$10^2$×6,2، $10^2$ ×2.8± $10^2$ ×7,1 و $10^2$ × 0,4±$10^2$×1,2 علي التوالي . كما تم فحص 10 معزولات للميكروب لاختبار مدي قدرتها علي افراز السموم المعويه A,B,C, D , E فوجدت معزولاتان ايجابيتان لافراز السم المعوي A واحده من عينه جبن قريش والاخري من عينه جبن تلاجه وايضا معزولاتان ايجابيتان لسموم المعويه A, C معزولاتان من عيناتان للجبن القريش . هذا وقد تم فحص نفس عينات الجبن للكشف عن تواجد تلك السموم في العينه نفسها فكانت النتيجة سلبيه.

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