

## Original Research Article

ISSN (Print) 2786-0272 ISSN (Online) 2786-0280

## Incidence of Staphylococcus Aureus and Enterotoxin A Gene in Marketable Milk and Some Milk Products Sold in New Valley Governorate, Egypt.

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Submitted 3/5/2022

Accepted 3/24/2022

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### Abstract

*Staphylococcus aureus* (*S. aureus*) represents one of the most common pathogens responsible for food contamination worldwide. The existing work was scheduled to study the occurrence, count, and identification of *S. aureus* and investigation of the obtained isolates for presence of enterotoxin A encoding gene (*sea*) in milk and some milk products in New Valley Governorate, Egypt. A total of 150 irregular samples of marketable milk and some milk products including: soft cheeses (Kareish, Domiati) and ice cream (30 for each) were collected from different localities in New Valley Governorate. *S. aureus* occurred in 11.6, 10, 3.3 and 10% for marketable milk, kariesh cheese, domiati cheese and ice cream, respectively. The existence of the enterotoxin sea gene was detected in 4 (57%) and 2 (50%) in milk and soft cheese, respectively, while none of the isolates obtained from ice cream samples harbored this gene. The investigation revealed that the examined milk samples and its products were infected with *S. aureus* with average counts of  $2.8 \times 10^8$ ,  $7.5 \times 10^5$ ,  $7.6 \times 10^6$  and  $1.7 \times 10^7$  for milk, Kariesh cheese, Domiati cheese and ice cream samples, respectively. In conclusion, the presence of *S. aureus* in milk and some milk products indicated that the tested samples were of poor quality which reflect unsanitary practices during milking, processing, storage, and distribution chain.

**Keywords:** Enterotoxin A, Domiati cheese, Kareish, Milk, *Staphylococcus aureus*

### Introduction

Pathogen-induced food poisoning is a major public health concern worldwide with countries spending a lot of resources to overcome it. Bacterial food contamination is a source of concern for both industrialized and poor countries. (Ehuwa et al., 2021). Milk and its products are one of the most common food sources in the human diet and are directly available for consumption. However, it is known as a medium for different pathogens such as *Salmonella*, *S. aureus* and *Campylobacter* spp. (Ebraheem, 2011). Dairy products, such as cheese and ice cream, are contaminated with diverse bacteria from various sources during manufacture, processing, and handling that makes them inappropriate for consumption. (El-Kholy et al., 2016).

*S. aureus* are Gram-positive spherical bacteria that grow in tiny clusters like grapes. *S. aureus* is a common human pathogen and found on skin, abscesses, wound infection and normal inhabitant of nose and throats. *S. aureus* can adapt to different diets and produces food poisoning by secreting enterotoxins. Staphylococcal enterotoxins (SEs) are members of a family called: pyrogenic toxin superantigens (PTSAgs). The Traditional enterotoxins A, B, C, D, and E are poisonous proteins that can withstand temperatures of up to 100°C for several minutes, improperly prepared food contaminated with bacterium or its preformed toxins in sufficient concentrations ( $1 \times 10^5$ ) can cause staphylococcal food poisoning within a few hours (Letertre et al., 2003). Milk and dairy products are suitable for enterotoxin

formation because they encourage the growth of *S. aureus*.

It is worth noting that the presence of *S. aureus* in milk and dairy products, even in low concentrations, should be considered a public health risk because it has been demonstrated that they may lose viability in food, but enterotoxin still exists. The viability of *S. aureus* in dairy products, such as cheese, is determined by the presence of starter culture, salt content, and storage time. The syndrome of *staphylococcal* food poisoning is marked by nausea, vomiting, diarrhea, general malaise, weakness, severe abdominal pain, headache, muscular cramp and decrease of blood pressure; such symptoms appear within 24 hrs post-ingestion of toxic food (Carroll et al., 2012). Therefore, the present work was planned to study the occurrence of *S. aureus* and enterotoxin A in marketable milk and some milk products in the New Valley governorate in Egypt.

## Materials and Methods

### 1. Collection and preparation of samples

A total of 150 random samples of marketable milk from dairy farms and dairy shops (30 samples each) and some milk products including: soft cheeses (Kareish and Domiati) from grocery shops (30 samples each) and ice cream from street vendors and small-scale producers (15 samples each) have been collected from different localities in New Valley Governorate. These samples were collected in clean, dry, and sterile containers. The collected samples were sent to the laboratory as soon as possible to be tested. Samples were prepared as prescribed by (A.P.H.A., 2004), where One ml of well mixed milk sample was aseptically transferred to 9 ml of sterile 0.1 % peptone water solution and well mixed to obtain 1/10 dilution, then 10-fold serial dilution were prepared. Regarding cheese samples, it was mixed in a highspeed blender at room temp. not exceeding 24°C. 10g were mashed thoroughly in a sterile sand and transferred to sterile flask. Then 90 ml sterile saline was added (receive apart of sterile saline to rinse the mortar), then 2% sodium citrate were added to make dilution 1/10 and complete 10-fold serial dilution were prepared. Finally, ice cream was melted in container that placed in water bath at 40°C for 10 min. to prevent the multiplication of micro-organisms. Then 10 gm of melted ice cream has been added to 90 ml sterile saline or sterile peptone water to make a dilution of 1/10 and 10- fold serial dilution was prepared.

### 2. Enumeration of *S. aureus* from milk and milk products

From each dilution 0.1 ml was distributed over a dry surface of Baird-Parker plates using surface plating technique. Baird-Parker Agar plates were incubated for 24 hours at 37 °C. *S. aureus* was identified as black, glossy, with narrow white edges, and surrounded by transparent zones extending into the dark medium. The number of suspected colonies in countable plates was counted and *S. aureus* count per ml or g was computed and recorded. (Baer et al., 1971)

#### 2.1. Isolation of *S. aureus*

Ten ml or g of the prepared samples of milk or milk products were inoculated into a sterile test tube containing sterile Sod. chloride broth 10% and incubated at 37°C for 24 hrs. Then, a loopful from the incubated Sod. chloride broth tubes are streaked into mannitol salt agar plates in a manner to obtain separate colonies and incubated at 37°C for 24 h. Suspected colonies were picked up onto tryptose soya agar (TSA) slants and incubated at 37°C for 24 hrs. *S. aureus* on mannitol salt agar plates showing yellow colonies surrounded by bright yellow zone. (Dai et al., 2019). Isolates were confirmed using catalase test, coagulase test and anaerobic utilization of mannitol tests according to (Zhang et al., 2016).

### 3. Polymerase chain reaction (PCR)

#### DNA extraction

Suspect colonies and control strains were subcultured onto nutrient broth and incubated at 37°C overnight. Qiagen DNA Blood Mini kit (Cat. No. 51104, Hilden, Germany) was used to extract DNA, and the isolated DNA was stored at -20°C until further investigation.

#### DNA amplification

The *S. aureus* and its enterotoxin genes were examined using sea primer for 16S rRNA, PCR reactions were performed in final volume of *S. aureus* 10µl including 5µl master mix (Green Master, Promega, USA), 0.5µl primer (F and R), 1ng of DNA template and 3µl nuclease free water. The annealing temperature was 52°C (Monday et al., 1999).

The amplification was carried out in a programmable thermal cycler (Gradient Thermal Cycler, Veriti Applied Biosystem, USA) for 10 minutes at 95°C, followed by 40 cycles of denaturation. Denaturation at 94°C for 50 sec, and the annealing temperature were set to each primer (Table-1) followed by extension at 72°C for 1 min and final extension at 72 °C for 10 min. The PCR products were preserved at 4°C.

**Table 1.** Primer sets that are used to amplify fragments of 16S rRNA, sea enterotoxins gene in *S. aureus*.

Primer name	Sequence	Amplified product size (bp)	Annealing Temp.
16S rRNA	GTA GGT GGC AAG CGT TAT CC CGC ACA TCA GCG TCA G	228	52
Ref.	Monday et al., 1999		
Sea	TTGGAAACGGTTAAAACGAA GAACCTTCCCATCAAAAACA	120	55
Ref.	Johnson et al., 1991		

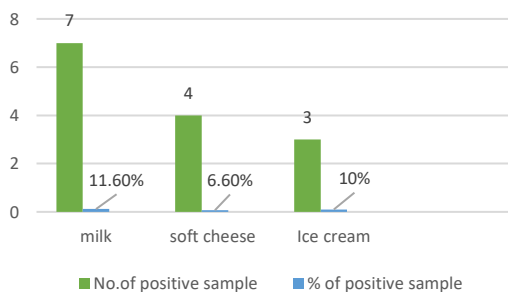
**Gel electrophoresis**

PCR products were electrophoresed at 100 volts for 60 minutes in a 1% agarose gel (GX 040.90, Gen Agarose L.E., Standard DNA/RNA agarose, Molecular Biology Grade, Inno-Train Diagnostic, D-61476, Kronberg/Taunus) containing 10% diluted ethidium bromide 1 l/ml electrophoresis buffer. In SCiE-PLAS, HU 10, 5636, UK, a 100 bp DNA ladder was used. The gel was next examined using a high-performance ultraviolet (UV) transilluminator (UVP Inc, UK) and the image acquisition program DOC-It® LS (UVP Inc., UK).

**Results**

**Table 2.** Incidence and loads of *S. aureus* in the examined marketable milk and some milk products samples.

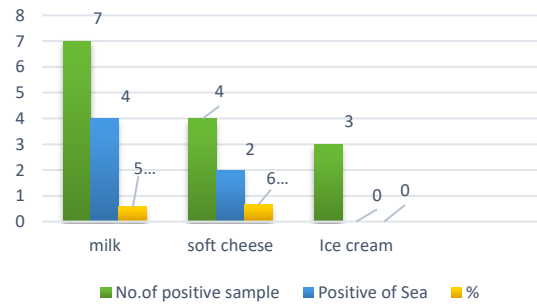
Sample type	No. Samples	Positive		Count/ml or g of +ve samples		
		No.	%	Min.	Max.	Average
Milk	60	7	11.6	1.09×10 <sup>7</sup>	3×10 <sup>8</sup>	1.2×10 <sup>8</sup>
Kariesh cheese	30	3	10	2.1×10 <sup>4</sup>	2.9×10 <sup>6</sup>	9.8×10 <sup>5</sup>
Domiaty cheese	30	1	3.3	>10 <sup>2</sup>	>10 <sup>2</sup>	>10 <sup>2</sup>
Ice cream	30	3	10	4×10 <sup>5</sup>	2.08×10 <sup>7</sup>	7.2×10 <sup>6</sup>



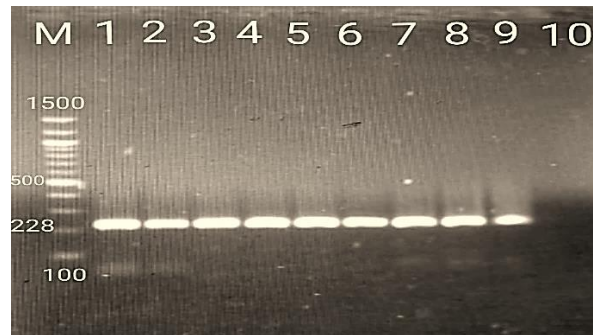
**Figure 1.** Incidence and loads of *S. aureus* in the examined marketable milk and some milk products samples.

**Table 3.** Frequency of distribution sea of *S. aureus* in the examined marketable milk and some milk products samples.

Examined sample	No. of positive sample	Positive of sea	%
Marketable milk	7	4	57.14%
Kariesh cheese	3	2	66.66%
Domiaty cheese	1	--	0
Ice cream	3	--	0
Total	14	6	42.85%

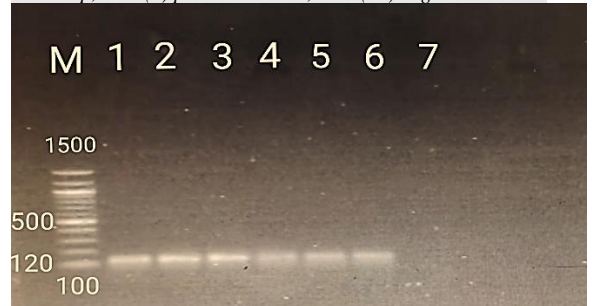


**Figure 2.** Frequency of sea of *S. aureus* in the examined milk and some milk products samples.



**Photo 1.** PCR identification of *S. aureus* using 16SrRNA.

\*Implications of the gene 16S rRNA gene of *S. aureus*. Lane (M) DNA ladder 100 bp, lanes (1-8) positive samples of 16S rRNA gene at 228 bp, lane (9) positive control, lane (10) negative control.



**Photo 2.** Agarose gel electrophoresis of MPCR of sea gene.

\*Implications of the gene sea. Lane (M) DNA ladder 100 bp, lanes (1-6) positive samples of sea gene at 120 bp and lane (7) negative control.

**Discussion**

In this study, the prevalence of *S. aureus* was investigated in various samples collected from New Valley governorate (150 samples of milk, soft cheese, and ice cream) and enterotoxin A encoded by *sea* gene was detected in the obtained isolates. According to the data recorded in Table 1, it is clear that the examined milk samples were contaminated with *S. aureus* with average count 1.2×10<sup>8</sup> cfu/g and showed that kariesh cheese samples were contaminated with *S. aureus* with average count 9.8×10<sup>5</sup> cfu/g, and ice cream samples were contaminated with *S. aureus* with average count

$7.2 \times 10^6$  cfu/g. Similar results were recorded by Hassan and Gomaa (2016). The lower counts were recorded by Abd Elall et al. (2006); Wafy et al. (2006) Hassan et al. (2008); El-Zamkan (2010); Sayed et al. (2011); El-Malt et al. (2013), Eid and Eltalawy (2014); El-Kholy et al. (2014) and Elshall (2019).

According to Table 1, the incidence of *S. aureus* were 16.6 and 6.6% of the tested marketable milk sample from dairy farm and dairy shops, respectively. The incidence of *S. aureus* in the total examined milk samples were 11.6%. Nearly similar result was recorded by China Health (2015). These results were extremely higher than those obtained by Dai et al. (2019). On the other side the lower results were reported by Ali et al. (2011); Emam et al. (2021); Yahya et al. (2021) and Zeinhom and Abed. (2021).

The obtained results in Table 1, showed that the incidence of *S. aureus* in the examined soft cheese samples. The incidence were 10 and 3.3% of the examined Kariesh cheese and Domiati cheese, respectively. The total percentage of *S. aureus* in the examined soft cheese samples were 6.6%. Nearly similar result was recorded by Sayed (2020); these results were higher than those carried by Ghada et al. (2002) and Elbassiony et al. (2021). In addition, these results were lower than those obtained by El-Malt et al. (2013); Eid and Eltalawy (2014); Elshall (2019) and Zeinhom and Abed. (2021).

It was clear that the heavy contamination of samples may be due to contamination during milking and unsanitary storage, handling, and packaging. Also, the presence and growth of *S. aureus* in cheese indicate contamination of milk from diseased udder or any external sources such as dairy animals, hands, sneezing and coughing of dairy workers or may be refer to insufficient heat treatment, unsanitary handling techniques, the use of filthy containers, and poor storage and transportation.

Regarding ice cream samples, the incidence of *S. aureus* was 10% of the tested samples (Table 1). These results were higher than those obtained by Gücükoğlu et al. (2013). While the obtained results were lower than those obtained by Mirzaei et al. (2012); Abo EL-Makarem, (2017) and Samir et al. (2018). The presence of *S. aureus* in ice cream demonstrates the inadequate quality and safety of the ingredients used in its production. In addition to inadequately cleaned equipment, air incorporation, product recirculation, and personnel, post pasteurization contamination causes include fluid, dry components, as well as the addition of flavor, coloring agents, fruits, nuts, and chocolate chips to the mix. (El-Kholy et al., 2016).

Screening *S. aureus* isolates for *sea* encoding enterotoxin A gene revealed that 6(42%) of the obtained isolates from marketable milk and some milk products samples carried this gene (Table 2). Enterotoxin *sea* gene was in 4 (57%) in marketable milk samples isolates, while in soft cheese samples isolates *sea* gene was detected in 2 (50%). Contrarily, the *sea* gene was not detected in ice cream isolates. These results were extremely higher than those obtained by Gücükoğlu et al. (2013) and Mirzababaei et al. (2021). However, the obtained results were lower than those carried by Younis et al. (2021). The presence and growth of *S. aureus* in milk and some milk products represent a potential public health hazard since many strains of *S. aureus* can create thermostable enterotoxins that cause food poisoning intoxication. Toxin synthesis requires a large number of microorganisms,  $10^5$  or more, to begin food poisoning Mahmoud. (2020). Staphylococcal enterotoxins are known agents of staphylococcal food poisoning syndrome and may be involved in other forms of infections with shock sequelae in people and animals. Particularly Staphylococcal enterotoxin (SEA) is one of the most common causes of gastroenteritis. In some areas, Staphylococcal enterotoxin is responsible for more than half of all food poisoning A (*sea*). This microorganism's principal habitat is the nasopharyngeal mucosa and the skin of humans and animals Zargar et al. (2016).

## Conclusion

From the obtained results milk and some milk products such as Kariesh cheese, Domiati cheese and Ice cream are the vehicles for transamination of some food poisoning microorganisms such as *S. aureus* to humans. So, it is recommended to improvement of farm hygiene and conducting animal health programs to ensure the freedom of dairy animals from any microorganism during milk production, high quality milk production under the most possible hygienic conditions should be used with proper cleaning and sanitizing of all dairy utensils, proper cleaning and sanitizing of all milking equipment, utensils and all contact surfaces as a routine work, educational programs should be imposed for milk producers and handlers to improve the quality of milk and ensure safety to consume, application of good manufacture practices (GMPs) in milk farms, application of new quality assurance programs such as "HACCP" system which must be adopted in both milk production units and transportation and displaying the final products and Strict application of the "General Principles of Food Hygiene" issued by the "Codex Alimentarius

Commission" should be practiced in all chain of milk manufacturing.

### Conflict of interest

The authors declared no conflict of interest.

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