# OCCURRENCE OF ENTEROTOXIGENIC STAPHYLOCOCCUS AUREUS IN SOME CHEESE VARIETIES IN ASWAN CITY – UPPER EGYPT

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#### ABSTRACT

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A total of 120 random samples of some cheeses were collected from various dairy shops, street vendors and supermarkets located in Aswan city (upper Egypt) and The samples were examined microbiologically for enumeration. isolation and identification of Staph. aureus and detection of enterotoxigencity of the isolated Staph. aureus strains These samples included fresh Kareish, pickled Kareish, Domiati and Processed cheese (30 samples each).' Of the examined cheese samples 28(93%), 25(83%), 21(70%) and 6(20%), respectively were contaminated with Staph.aureus. With mean counts of 3.08 X  $10^6 \pm 1.79 \text{ x} 10^6$ , 3.40 x  $10^5 \pm 1.59 \text{x} 10^5$ , 7.24 x  $10^5 \pm 4.10 \text{ x} 10^5$  and 1.15 x10<sup>2</sup>±8.98, respectively. Incidence of coagulase positive and coagulase negative strains of S. aureus in the examined cheese samples were (15 (54%)) and (13 (46 %), (7 (28%) and (18(72%), (13(62%) and (8 (38%) and (0% and 6(100%), respectively. Methicillin resistant S. aureus (MRSA) was isolated in an incidences of 8(26%), 7(23%), 9(30%). and 0(0%) from examined cheese samples respectively while vancomycin resistant Staph aureus (VRSA) was isolated in an incidences of 3(10%), 5(17%), 8(27%) and 0(0%). Three out of twelve strains of MRSA and VRSA were isolated from fresh Kareish cheese 4 strains from Pickled Kareish cheese and 5 strains of Domiati cheese. Two strains produced enterotoxins A were isolated from fresh Kareish cheese which can also synthesis entertoxins B and another 2 strains isolated from Domiati cheese produce entertoxins A and also one strain synthesiss entertoxins B, entertoxins C, and enterotoxins D. The selected 12 strains were strongly produce coagulase only 4 strains were enterotoxigenic.

Key words: Staph.aureus, Enterotoxigenic, cheese.

## INTRODUCTION

Cheeses are ready to eat food products that do not undergo any further treatment to ensure their safety before consumption Although cheeses have been characterized as one of the safest food products by some authors (Little *et al.*, 2008). In 2006 the consumption of contaminated cheese accounted for the 0.4% of the total food borne outbreaks in Europe (European Food Safety Authority "EFSA", 2008), furthermore, the scientific literature has reported severe food poisoning out breaks associated with various types of cheese. (Kongo *et al.*, 2008).

Staphylococcus aureus is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of Staphylococcal enterotoxins preformed in the food (Loir *et al.*, 2003)

Many contaminants find their way to raw milk, from which they gain access to dairy products (Al–khatib and Al-Mitwalli, 2009). Chapaval *et al.*, (2010) found *staphylococcal* enterotoxins in milk when milk was stored at temperatures of 37 °C to 42 °C or when exposed to variations in temperature.

On heating at normal cooking temperature, the bacteria may be killed but the toxins remain active (Presscott *et al.*, 2002). Staphylococcal enterotoxins are highly heat resistant and are thought to be more heat resistant in foodstuffs than in a laboratory culture medium (Bergdoll 1983).

Besides enterotoxins producing S. aureus are most dangerous and harmful for the human health about 50 % of this organism are able to produce enterotoxins associated with food poisoning (Payne, 1974). Illness through S. aureus ranges from minor skin infection such as pimples, boils, cellulites, toxic shock syndrome impetigo, and abscesses to life threatening

disease such as pneumonia, meningitis, endocarditis, and septicemia (Soomro *et al.*, 2003).

SEA and SED are the most common Ses involved in food poisoning outbreaks (Genigeorgis, 1989). Growth of enteroxigenic strains of Staph. aureus to a population of 10<sup>6</sup> or more cells/g of food is generally considered necessary for production of a sufficient amount of enterotoxins to cause intoxication if the food consumed (Newsome, 1988). The amount of SEA necessary to cause symptoms in humans is about 100ng (Balaban and Rasooly, 2001). The Ses functions as potent gastrointestinal toxins. After ingestion of contaminated food, the toxins are resorbed into the blood in the gastrointestinal tract, activate an emetic reflex and cause nausea, emesis, abdominal cramps an diarrhea (Tortora, 1995) after 2-6 hours of eating contaminated food and the recovery takes 1-3 days, while deaths occur rarely, specifically in very young or old age (Martin and Iandolo, 2000) Time of onset and severity of symptoms depend on the quantity of toxins consumed and the individuals susceptibility (Baird and Lee, 1995).

To detect the level of enterotoxin (100ng) several sensitive detection methods for SE detection have been developed in particular the rapid methods based on specific antibodies such as a radioimmunoassay (RIA) an enzyme linked immunsorbent assay (ELISA) or enzyme immunoassay (EIA) and reversed passive latex agglutination assay (RPLA) are commonly used in staphylococcal intoxication the use of polymerase chain reaction (PCR) and DNA probes is limited in detection only the presence of *Staph aureus* genes, but not in detection the presence of preformed toxins (Di Pinto *et al.*, 2004).

Antimicrobial resistance is a major public health problem in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of bacteria in the environment and the possible contamination of water and food. The development of resistance both in human and animal bacterial pathogens has been associated with the extensive therapeutic use of antimicrobials or with their administration as growth promoters in food animal production (Barber *et al.*, 2003)

## The present study was planned to deal with the following:

- **1-**Enumeration, isolation and identification of *staph*. *aureus* in some cheese varieties sold in Aswan city.
- **2-** Identification of antibiotics resistant strains of *staph. aureus*.
- **3-** Detection of enterotoxigencity of the isolated *staph. aureus* organisms

## **MATERIALS and METHODS**

## A) Collection of samples:

A total of 120 random samples of some cheese varieties were collected from various dairy shops, street vendors and supermarkets located at Aswan city. These samples included Fresh Kareish, pickled Kareish, Domiati and Processed cheese (30 samples each). Collected samples were transferred in an ice box directly to the laboratory with a minimum of delay to be examined.

- B) Preparation of serial dilutions (ISO 8261. 2001):
- **C)** Microbiological examination:
- **1-** Enumeration and isolation of *Staph. aureus* (*A*.O.A.C., 2000):

Over duplicated plates of a dry surface of Baird Parker (B-P) agar, 0.1 ml from each prepared dilutions of examined samples were transferred and evenly spread using surface plating technique (Thatcher and Clark, 1988).

- The inoculated B-P agar plates were incubated at 37°C for 24-48 hrs. Suspected colonies are circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet black, shiny, with light color (off-white) narrow margin, surrounded by hallow zones and had buttery to gummy consistency when touched with inoculating needle were counted. The plates were then reincubated for additional 18-24 hrs before being counted for further growth, *Staph.* aureus count/ g were recorded.
- Furthermore, an appropriate amount from each prepared sample was inoculated into sterile Nacl 10% broth. Inoculated Nacl 10% broths were inoculated at 37 °C for 24 hrs. A loopful of the incubated broth was streaked onto sterile plates of Mannitol Salt agar. (A.O.A.C., 2000)

Identification of *Staph. aureus* recovered from the examined samples:

- **A-** Morphological characters for all isolates:
- 1- Staining reaction (A.P.H.A., 2004):-
- **B-** Biochemical reactions:- catalase activity (Koneman *et al.*, 2005), anaerobic mannitol fermentation (Baird-Parker, 1962), coagulase test. according to (Cruickshank *et al.*, 1973).
- **2-** Isolation of antibiotics resistant strain of *staph. aureus*.
- **a-** Identification of methicillin resistant *Staph aureus* (MRSA): (Simor *et al.*, 2001).

The cultures of *Staph aureus were* subcultured on Oxacillin Resistance Screen Agar Base (ORSAB) (Oxoid Limited, Basingstoke, England) containing ORSAB Selective Supplement contained two antibiotics-oxacillin at 2mg/L and polymyxin B

50.000 IU/l. The plate was incubated at 37 °C for 24-48 h and examined for the presence of MRSA colonies, which were blue on ORSAB.

**b-** Identification of vancomycin resistant *Staph aureus* (VRSA) ⊗Tiwari and Sen, 2006).

**3-** Detection of the enterotoxigencity of Staph. aureus (Park *et al.*, 1994)

Using RIDASCREE®SET A, B, C, D, E (Art. No.:R4101) manufactured by R- Biopharm AG, Germany.

Selected strains of the isolated MRSA and VRSA from the examined samples were tested for –ability to produce entertoxins.

#### RESULTS

**Table 1:** Statistical analytical results of *Staph.aureus* count in the examined cheese samples.

Examined samples	No. of examined	Positive samples		Count /g				No. of samples above E.S.*	
	samples	No.	%	Min.	Max.	Mean	S.E.	No.	%
Fresh Kareish cheese	30	28	93	$1x10^3$	5.25x10 <sup>7</sup>	$3.08x10^6$	1.79x10 <sup>6</sup>	28	93
Pickled Kareish cheese	30	25	83	$4.17x10^2$	$4.70 \times 10^6$	$3.40 \times 10^5$	1.59x10 <sup>5</sup>	25	83
Domiati cheese	30	21	70	1x10	$1.27x10^7$	$7.24 \times 10^3$	$4.10 \times 10^5$	21	70
Processed cheese	30	6	20	1x10	$1.90 \times 10^2$	$1.15 \times 10^2$	8.98	6	20

<sup>\*</sup>E.S.: Egyptian Standard (2005).

**Table 2:** Frequency distribution of positive cheese samples based on their S.aureus count.

Count /g	Fresh Kareish cheese		Pichled Kareish cheese		Domiati cheese		Processed cheese	
	No./28	%	No./25	%	No./21	%	No./6	%
	-	-	-	-	-	-	-	-
$10^{1} >$	-	-	-	-	-	-	-	-
$10^{2}$	-	-	2	8	9	43	6	100
$10^{3}$	8	28.5	11	44	7	33	-	-
$10^{4} >$	6	21	2	8	8	14	-	-
$10^{5} >$	5	18	9	36	-	-	-	-
$10^{6->}$	8	28.5	1	-	1	5	-	-
$10^{7} >$	1	4	_	4	1	5	-	_

**Table 3:** Incidence of coagulase positive and coagulase negative strains of *S. aureus* in the examined cheese.

Examined samples	No. of	No. of isolated S.aureus	coagulase positive		coagulase negative	
	examined samples		No.	%	No.	%
Fresh Kareish cheese	30	28	15	54	13	46
Pickled Kareish cheese	30	25	7	28	18	72
Domiati cheese	30	21	13	62	8	38
Processed cheese	30	6	-	-	6	100

**Table 4:** Frequency distribution of MRSA and VRSA and recovered from the examined cheese samples.

Examined samples	No. of isolated S.aureus	MRSA		VRSA		Both	
	S. Wall Culb	No.	%	No.	%	No	%
Fresh Kareish cheese	28	8	29	3	11	3	11
Pickled Kareish cheese	25	7	28	5	20	4	16
Domiati cheese	21	9	43	8	38	3	14
Processed cheese	6	-	-	-	-	-	-

Table 5: Incidence of MRSA and VRSA strains isolated from the examined cheese

	No. of examined	MR	SA	VRSA	
Examined samples	samples	No.	%	No.	%
Fresh Kareish cheese	30	8	26	3	10
Pickled Kareish cheese	30	7	23	5	17
Domiati cheese	30	9	30	8	27

**Table 6:** Enterotoxins produced by some strains of *Staph. aureus* isolated from the examined cheese samples.

Examined	No. of No. of strains		Types of produced enterotoxins					
samples	strains tested	producing enterotoxins	A	В	С	D	Е	
Fresh Kareish cheese	3	2	2	1	-	-	-	
Pickled Kareish cheese	4	-	-	-	-	-	-	
Domiati cheese	5	2	2	1	1	1	-	

**Table 7:** Entertoxins production in relation to coagulase producing *Staph. aureus* strains.

No. of strains tested	Coagulase producing strains.		Enterotoxigenic Sta	aph. aureus strains
	No.	%	No.	%
12	12	100	4	33.3

### **DISCUSSION**

The results recorded in Table 1showed that 28(93%), 25(83%), 21(70%) and 6(20%) of the examined fresh Kareish, Pickled Kareish, Domiati and processed cheese samples were contaminated with *Staph.aureus*, respectively.

The *S.aureus* count ranged from  $1 \times 10^3$  to  $5.25 \times 10^7$  with a mean count of  $3.08 \times 10^6 \pm 1.79 \times 10^6$  in fresh Kareish cheese samples, from  $4.17 \times 10^2$  to  $4.70 \times 10^6$  with a mean Count of  $3.40 \times 10^5 \pm 1.59 \times 10^5$  in Pickled Kareish cheese samples, from  $1 \times 10$  to  $1.27 \times 10^7$  with a mean count of  $7.24 \times 10^5 \pm 4.10 \times 10^5$  and for Processed cheese the counts ranged from  $1 \times 10$  to  $1.90 \times 10^2$  with a mean count of  $1.15 \times 10^2 \pm 8.98$  cfu/g.

Egyptian standards (2005) pointed out that the cheese must be free from Staph. aureus and its toxins It was evident that 28(93%), 25(83%), 21(70%) and 6(20%) of the examined fresh Kareish, Pickled Kareish Domiati and processed cheese samples failed to comply with the standard, respectively.

The results in Table 2, revealed that the highest frequency distribution of fresh Kareish cheese samples was 8(28.5%) lied in the range of  $10^3$  < $10^4$  and  $10^6$  < $10^7$  cfu/g, while the rest of the positive samples were distributed as 21,18 and 4% lied in between  $10^4$  < $10^5$ ,  $10^5$  < $10^6$  and  $10^7$  < $10^8$  cfu/g, respectively. In case of Pickled Kareish cheese samples the highest frequency distribution was (44%) lied in the range of  $10^3$  < $10^4$  cfu/g, while the rest of the positive samples were distributed as 36,8,8 and 4% lied in between  $10^5$  < $10^6$ ,  $10^2$  < $10^3$ ,  $10^4$  - < $10^5$  and  $10^7$  < $10^8$  cfu/g, respectively.

For Domiati cheese samples the highest frequency distribution was (43%) lied in the range of  $10^2 < 10^3$ cfu/g, while the rest of the positive samples were distributed as 33, 14 and 10 % lied between  $10^3 < 10^4$ ,  $10^4$  <105 and  $10^6$  <10<sup>7</sup>, respectively. While the Processed cheese positive samples all of them lie in range 10<sup>2</sup> <10<sup>3</sup> fresh Kareish cheese samples comparatively higher counts were obtained by Tawfek et al. (1988) Relatively lower counts were detected by Shelaih (1979), Ahmed (1980). Ewida (2009), De Reu et al. (2002) and In case of the samples examined Pickled Kareish cheese comparatively lower counts were obtained by Kaldes (1997). While for Domiati cheese samples results, higher counts obtained by Sallam et al. (1985). Relatively lower counts obtained by El-Malt (1993) and Hassan (2009). Lower incidence of S.aureus in curd (6.66 %) was reported by Kumar and Prasad (2010) and (3.33 %) Thaker et al. (2013). The difference in the prevalence of S.aureus between different types of cheese may originate from the method of manufacture, storage and handling.

Turkoglu *et al.* (2001) and Soncini *et al.* (2002) failed to detect the S. aureus in the examined Processed cheese samples.

Fresh Kareish cheese is a popular Egyptian food. It is made at farmers houses from raw skim milk and the fresh product is either consumed fresh or consumed after pickling in its salted fresh whey with added spices and medical plants (mish cheese) so the source of Staph.aureus in Kareish cheese are the raw milk, during processing and distribution in village markets these sources lead to the expected high count of Staph. aureus. Domiati cheese is considered to be the main national popular pickled soft cheese produced in Egypt. It is traditionally prepared from raw milk without addition of lactic acid culture starters but by addition of appreciable amount of salt to milk before renneting. It can be consumed fresh, but usually it is consumed after pickling in its salted fresh whey for a period of not less than 8 weeks, the product may be soiled with pathogens which enter through different environmental sources that render it a source of infection by many diseases. Genigeorgis (1989) demonstrated that the higher concentration of competing microorganisms in milk, the lower the rate of S. aureus growth and SE production. Competition with lactic acid bacteria has been reported in other research on cheese (Otero et al., 1988).

Table 3 showed that 15(54%), 7(28%) and 13 (62 %) of the examined staphylococci strains recovered from fresh Kareish cheese, Pickled Kareish cheese and Domiati cheese, respectively. were coagulases positive. While, 13(46%), 18(72%), 8(38%) and 6(100%) of the isolated strains of staphylococci of the examined cheese, respectively. were coagulase negative. Concerning the result of coagulase positive *S.aureus* in the examined fresh Kareish cheese samples, higher results were obtained by Ahmed (1980), Al-Hawary *et al.* (2009) and Helmy *et al.* (2009). Lower results were detected by Aman (1994); Kaldes (1997) and Kolluman *et al.* (2011).

In case of coagulase positive *S.aureus* in the examined Domiati cheese samples, lower results were detected by Coveney *et al.* (1994), Kaldes (1997); Normanno *et al.* (2005) and Kolluman *et al.* (2011).

Various examples of staphylococcal food poisoning are described in the literature. In one case, cheese was involved in an outbreak because it had been made from unheat treated milk, milk contaminated after pasteurization and before inoculation with lactic starter culture or did not use culture starter. In this particular case, the starter culture did not grow properly, resulting in a fermentation accident that allowed the S. aureus strain to develop and produce SE (Vasavada, 1988) Although milk and milk products are frequently contaminated with S. aureus, dairy products are rarely involved in staphylococcal

food poisoning because the critical cell density of >105 cfu/g-1 is usually not reached (Altekruse *et al.*, 1994).

The result in Table 4 showed that 8(29%), 3(11%) and 3(11%) showed *staph aureus* strains isolated from fresh Kareish cheese were MRSA, VRSA, respectively. While 7(28%), 5(20%) and 4(16%) *Staph aureus* strains isolated from Pickled Kareish cheese were MRSA, VRSA, respectively. The frequency distribution of MRSA and VRSA in the Domiati cheese samples were 9(43%) and 8(38%) while 3(14%) can be resistant for both drugs. In Processed cheese there is no of isolated strains of MARSA, VRSA or resistant for both drugs.

In Table 5 the incidences of MRSA and VRSA from the examined samples were 26%, 3(10%), 7(23%), 5(17%), 9(30%) and 8(27%) of fresh Kareish cheese, Pickled Kareish cheese and Domiati cheese respectively.

Inspection of Table 6 revealed that out of 12 methicillin and vancomycin resistance *Staph aureus* strains tested for enterotoxins production 3 strains of fresh Kareish cheese, 4 strains of Pickled Kareish cheese and 5 strains of Domiati cheese. From fresh Kareish cheese 2 strains were synthesized enterotoxins A and SEB (one strains) in Pickled Kareish cheese there is no strains producing interotoxins. Domiati cheese 2 strains were isolated and produce enterotoxins A(2 strains), SEB (one strain), SEC (one strain) and SED (one strain).

This finding was in contrast to other studies from Spain, Kenya, Switzerland, Brazil, South Korea, the USA, Slovakia and Palestine, where most of the enterotoxigenic *Staph aureus* isolated usually synthesized the toxins SEA, SEC, or SED (Scherrer *et al.*, 2004).

The higher percentages of SEA among Staph aureus strains isolated from milk and milk products may be due to the fact that enterotoxins A are less common among the strains of animals origin than from human origin (Hajek and Marsalek, 1973). These strains of human origin contaminate milk and milk producte during different stages of production or at consumer outlet. On the other side the presence of SEC and SED can be attributed to the increased incidence of *Sraphylococcal* mastitis as enterotoxins C and D were found to be produced by Staph aureus strains isolated from bovine mastitis and were designated as "animal strains" (Olson *et al.*, 1970).

Although the selected 12 isolates were strongly producing coagulase, only 4 were enterotoxigenic which confirm what was stated by A.P.H.A. (1992) and Ryser (2001) Table (7) that attempts to associate enterotoxin production by *Staph aureus* with specific

biochemical properties were generally failed. Consequently, confirmation of the toxin by serological or other means provide the only proof that the particular strain is enterotoxigenic.

Park et al. (1994) evaluated the RIDASCREEN SET kit for its efficiency. They concluded the major advantages of the kits were a high degree of specify (neither false –positive results due to the growth of nonstaphyloccal microorganisms nor cross –reaction among reagents of the kits was reported), excellent sensitivity, simplicity rapidity (results can obtained in 3h) and semi quantitative results.

## **CONCLUSION**

The obtained results allow concluding that fresh Kareish cheese, Pickled Kareish cheese, Domiati cheese and Processed cheese samples sold in Aswan city markets were produced, handled, packed and distributed under neglected hygienic measures. The information given by the achieved results proved that most of the examined fresh Kareish cheese, Pickled Kareish cheese, Domiati cheese and Processed cheese samples sold in Aswan city market were highly contaminated with high number of Staph aureus which may lead to undesirable changes of these products that render them unfit for consumption and indicate unpersonal hygiene and un-sanitary conditions during processing and handling of such product in compared with examined Processed cheese which show lower incidences or counts.

In the resent years, the extensive therapeutic use of antimicrobials or with their administration as growth promoters in food animal production lead to development of resistance both in human and animal's pathogens as occurred with *Staph aureus*. Nowdays, *Staph aureus* can resist many antibiotics as methicillin and vancomycin which lead to difficulty in their susceptibility of *Staph aureus to these antibiotics and other group of antibiotics. In this study, methicillin resistance* Staph aureus (MRSA) and vancomycin *resistance* Staph aureus (VRSA) could be isolated from examined samples in different percentages.

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## تواجد المكور العنقودي الذهبي المفرز للسموم المعوية في بعض الجبنة المباعة بمحافظة أسوان مصر العليا

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يعتبر الميكروب المكور العنقودي الذهبي من أهم الميكروبات التي تساهم في احداث حالات التسمم الغذائي التي اهم اعراضها الاسهال وما زال يسبب الكثير من المشاكل الصحية الاخرى على مستوى العالم مثل تسمم الدم البكتيري والتهاب عضلة القلب والتهاب العظام هذا وتعتبر الالبان ومنتجاتها من اهم الاغذية التي يمكن ان يصل اليها الميكروب حيث انه بعيش في الامعاء والجهاز التنفسي وعلى جلود الانسان والماشية وكذلك أيضاً يوجد في ضرع الماشية ويسبب الالتهاب الضرعي ولذلك يكون من السهولة تلوث اللبن به أجريت الدراسة على عدد مائة وعشرين عينة من الجبن المصنع محليا ثلاثون عينة من كل من (الجبن القريش الطازج ، الجبن القريش المملح ، الجبن الدمياطي)، والجبن المطبوخ المصنع في شركات الالبان وقد جمعت العينات من الباعة الجائلين وأسواق مدينة أسوان بطريقة عشوائية لفحصها وقد تبين بـالفحصّ البكتريولجي أن نسبة تواجد الميكروب المكور العنقودي الذهبي ٢٨(٩٣%) ، ٥٢ (٨٣)، ٢١ (٧٠%) و ٦ (٢٠٠%) لكل من الجبن القريش الطازج ، الجبن القريش المملح ، الجبن الدمياطي والجبن المطبوخ على التوالي. وكان متوسط العدد الكلي للمكور العنقودي الذهبي لهذه المنتجات على التوالي  $ilde{\mathsf{M}}$  .  $ilde{\mathsf{M}}$ ا الميكروب المكور العنقودي  $^{\circ}$  . ا  $^{\circ}$   $^{\circ}$  . ا  $^{\circ}$  . I  $^$ الذهبي المقاوم للمثيسيللين من العينات المفحوصة بنسبة ٨(٢٦%) ، ٧(٣٠%) ، ٩(٣٠%) و ٠(٠%) على الترتيب بينما تم عزل الميكروب المكور العنقودي الذهبي المقاوم للفانكوميسين بنسبة ٣(١٠/%)، ٥(١٧/%)، ٨(٢٧%) و ١٠(٠) من العينات المفحوصة على التوالي. وباستخدام اختبار ELISA تم تصنيف السموم المفروزة من اثني عشرة عترة من الميكروب المكور العنقودي الذهبي المقاوم للمتيسيللين وللفانكوميسين (ثلاثة عترات من جبن القريش، واربعة عترات من الجبن القريش المملح وخمس عترات من الجبن الدمياطي) وقد وجد ان اربعة عترات من (عترتين تم عزلهم من جبن القريش وعترتين تم عزلهم من الجبن الدمياطي) لهم قدرة على افراز سم A وتم عزل عترة واحدة من جبن القريش لها القدرة على افراز سم B وكذلك عترة من الجبن الدمياطي تفرز ثلاث انواع B. C&D من السموم