

## **OCCURRENCE OF *BACILLUS CEREUS* AND *STAPHYLOCOCCUS AUREUS* ORGANISMS IN SOME DAIRY DESSERTS**

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### **ABSTRAC**

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A total of 60 dairy desserts samples comprising ice cream, mehallabia and rice with milk (20 samples of each) were collected from different dairy shops and supermarkets in Assiut city, Egypt. All samples were examined bacteriologically for isolation and enumeration of *B. cereus* and *Staph. aureus* organisms. The incidences of *B. cereus* in ice cream, mehallabia and rice with milk samples were 55, 60 and 15%, and the average counts were  $3.1 \times 10^9$ ,  $1.07 \times 10^{10}$  and  $1.7 \times 10^9$  cfu/g food, respectively. The incidence of *Staph. aureus* in this study was 15% for each of ice cream and rice with milk samples with average of  $6.7 \times 10^5$  and  $2.7 \times 10^7$  cfu/g food, respectively. *Staph. aureus* organisms could not be detected in all examined mehallabia samples in this study. *Staph. aureus* enterotoxins A and C were detected in some food positive samples for staphylococcal isolation. The public health importance of the isolated organisms was also discussed.

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**Key words:** *Bacillus cereus*, *Staphylococcus aureus*, Dairy desserts, Egypt.

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### **INTRODUCTION**

Dairy desserts as ice cream, mehallabia (a traditional dessert in Egypt) and rice with milk are popular frozen and refrigerated foods consumed particularly in summer, as well as, throughout all the year. They continue to present a dominant interest for a large segment of population.

The ingredients of dairy desserts may be various combinations of milk, cream, evaporated or condensed milk, dried milk, coloring materials, flavors, fruits, nuts, rice, starch, sweetening agents, eggs, eggs products, and stabilizers. One or more of these components may contribute microorganisms and affect the quality of the product as judged by its bacterial load or its content of various specific species of bacteria especially food poisoning ones.

*Bacillus cereus* has raised concern in dairy industry by its deteriorating effect, this because *B. cereus* is a psychrotrophic microorganism able to grow under chilling temperature and produce lipases and proteases enzymes that change the components of dairy products (Zhou *et al.*, 2010). Several *B. cereus* strains have been identified as the causative agent of two different types of food poisoning: The emetic type (Ehling-Schulz *et al.*, 2004) and the diarrheal

type, and both type can occasionally be fatal (Dierick *et al.*, 2005).

Staphylococcal intoxication, which is due to the consumption of food containing one or more preformed staphylococcal enterotoxins (SE), is one of the leading food-borne diseases worldwide (Balaban and Rasooly, 2000). *Staph. aureus* enterotoxins exhibit two separate biological activities; they cause gastroenteritis in the gastrointestinal tract and act as a superantigen on the immune system (Shinefield and Ruff, 2009).

As most of the dairy desserts consumers are children of vulnerable age groups, it is required to be microbiologically safe (Warke *et al.*, 2000 and Caglayanlar *et al.*, 2009). Therefore, the objective of this study was to isolate, quantify and identify *Bacillus cereus* and *Staph. aureus* organisms from some dairy desserts (ice cream, mehallabia and rice with milk) and detection of enterotoxins of *Staph. aureus* in these desserts consumed in Assiut city, Egypt.

### **MATERIALS and METHODS**

A total of 60 dairy desserts samples comprising ice cream, mehallabia and rice with milk (20 samples of

each) were collected from small dairies in Assiut city, Egypt. The samples were collected in its container as sold to the public and transported as soon as possible to the laboratory and subjected to the following microbiological examination:-

**1-Enumeration and isolation of *Bacillus cereus* according to Holbrook and Anderson (1980).**

**2-Enumeration and isolation of *Staphylococcus aureus* according to FDA (2001).**

**3-Demonstration of *Staph. aureus* enterotoxins in dairy desserts samples (which was performed in Food Analysis Center, Faculty of Veterinary Medicine, Benha University, Egypt) by ELISA according to Ewald (1988):**

Accurately, RIDASCREEN set C (Art No.: R4101, R-Biopharm AG, Darmstadt, Germany) is an enzyme immunoassay for the determination of *S. aureus* enterotoxins by using their definite kits.

According to the test kit manual, food sample was mixed in sterile buffer saline and then shaken for 15 minutes. After centrifugation for 10 minutes at 3500 r.p.m, sterile filtration of the supernatant was applied. An aliquot (100 µl per kit well) of this solution was used in the test. Further, the last well was represented as positive control. They were mixed gently and

incubated for one hour at room temperature (20-25°C) in the dark.

The liquid was dumped out of the wells into a sink to remove all of the remaining liquid from the wells. Therefore, the wells were then filled with 250 µl of washing buffer and the liquid was poured out again. The washing step was repeated 3 times to remove the unbound conjugate.

Subsequently, 100 µl of enzyme conjugate were added to each well and incubated for one hour at room temperature in the dark after mixing gently. The liquid was dumped out of the wells into a sink and the wells were each filled with 250 µl of the washing buffer. The liquid was poured out again and the wells were emptied to remove all of the remaining liquid. The washing step was repeated 3 times again.

Afterwards, 50 µl of substrate and 250 µl of chromogen solutions were added to each well. The solutions were mixed gently and incubated for 30 minutes at room temperature in the dark. Finally, 100 µl of the stop solution (1M H<sub>2</sub>SO<sub>4</sub>) were added to each well with gentler mixing.

By using ELISA, the absorbance was measured at 450 nm in an ELISA plate reader (ELX800, BioTek Instruments, Bad Friedrichshall, Germany). The results were calculated from standard curve.

## RESULTS

**Table 1:** Incidence and counts of *Bacillus cereus* in some dairy desserts.

Types of samples	Incidence		Counts (cfu/g) in +ve samples		
	No.	%	Minimum	Maximum	Average
Ice cream (No. 20)	11	55	2 X 10 <sup>6</sup>	3 X 10 <sup>10</sup>	3.1 X 10 <sup>9</sup>
Mehallabia (No. 20)	12	60	6 X 10 <sup>6</sup>	1.2 X 10 <sup>11</sup>	1.07 X 10 <sup>10</sup>
Rice with milk (No. 20)	3	15	6 X 10 <sup>6</sup>	5 X 10 <sup>9</sup>	1.7 X 10 <sup>9</sup>
Total (No. 60)	26	43.33	2 X 10 <sup>6</sup>	1.2 X 10 <sup>11</sup>	6.47 X 10 <sup>9</sup>

**Table 2:** Incidence and counts of *Staphylococcus aureus* in some dairy desserts.

Types of samples	Incidence		Counts (cfu/g) in +ve samples		
	No.	%	Minimum	Maximum	Average
Ice cream (No. 20)	3	15	1.9 X 10 <sup>3</sup>	2 X 10 <sup>6</sup>	6.7 X 10 <sup>5</sup>
Mehallabeia (No. 20)	0	0	0	0	0
Rice with milk (No. 20)	3	15	8 X 10 <sup>4</sup>	8 X 10 <sup>7</sup>	2.7 X 10 <sup>7</sup>
Total (No. 60)	6	10	1.9 X 10 <sup>3</sup>	8 X 10 <sup>7</sup>	1.38 X 10 <sup>7</sup>

**Table 3:** Types of enterotoxins detected in *Staph. aureus* positive dairy desserts samples.

Serial No. of tested samples	Products	Enterotoxins production	Typing of enterotoxins	<i>S. aureus</i> counts (cfu/g)
4	Ice cream	- ve	----	$7 \times 10^3$
7	Ice cream	- ve	---	$1.9 \times 10^3$
19	Ice cream	+ ve	A	$2 \times 10^6$
28	Rice with milk	+ ve	A & C	$8 \times 10^4$
33	Rice with milk	- ve	---	$8 \times 10^5$
35	Rice with milk	+ ve	A	$8 \times 10^7$

## DISCUSSION

Results reported in Table 1 revealed that, *B. cereus* was isolated from 55% of ice cream samples in counts ranging from  $2 \times 10^6$  to  $3 \times 10^{10}$  with an average count of  $3.1 \times 10^9$  cfu/ml. Nearly similar result (52%) was estimated by Wong *et al.* (1988). Lower results (4, 44, 38, 40, 19, 48, 16 and 4%) were found by Masud (1989); Saleh *et al.* (1993); Al-Ashmawy *et al.* (1996); Warke *et al.* (2000); Yaman *et al.* (2006); Hassan *et al.* (2010); Altaf *et al.* (2012) and Maryam *et al.* (2013), respectively. In contrary, higher result (62.7%) was obtained by Messelhäusser *et al.* (2010).

The high average count of *B. cereus* obtained in this study may be attributed to fraud of the product with starch which is a favorable medium for *B. cereus* organisms or due to insufficient heating during the product manufacturing.

Concerning mehallabia samples, 60% of tested samples were contained *B. cereus* with counts ranged from  $6 \times 10^6$  to  $1.2 \times 10^{11}$  with an average count of  $1.07 \times 10^{10}$  cfu/g (Table 1). Lower result (44%) was revealed by Al-Ashmawy *et al.* (1996). As no available literature could be traced dealing with the incidence of *B. cereus* in mehallabia, therefore, it was hard to discuss the aforementioned result.

With regard to rice with milk, 15% of samples were contaminated with *B. cereus* in counts ranged from  $6 \times 10^6$  to  $5 \times 10^9$  with an average value of  $1.7 \times 10^9$  cells/g (Table 1). Lower result (5.8%) was estimated by Çadirici *et al.* (2013). On the other hand, higher results (64, 60 and 35%) were noticed, respectively, by Al-Ashmawy *et al.* (1996); Sadek *et al.* (2006) and Reyes *et al.* (2007).

From Table 1, one can easily conclude that, mehallabia samples had higher *B. cereus* incidence and counts than ice cream and rice with milk. This could be attributed to that main constituent of mehallabia is starch which is the main medium for *B. cereus* growth. Furthermore, rice with milk had the

lowest *B. cereus* incidence and counts in this study and this could be referred to over heating during rice with milk manufacturing or due to other factors which need further investigation.

From estimated results in Table 1, it is obvious that, the *B. cereus* containing samples were having *B. cereus* counts that sufficient enough to cause food poisoning as the infective dose of the microorganism ranged from  $10^3$  to  $10^{10}$  cfu/g (Notermans and Batt, 1998).

The results recorded in Table 2 revealed that, *Staph. aureus* was detected in 15% of ice cream samples in counts ranging from  $1.9 \times 10^3$  to  $2 \times 10^6$  with an average count of  $6.7 \times 10^5$  cfu/ml. Relatively similar result (18.7%) was observed by Mathews *et al.* (2013). Lower results (4.3, 4.4, 10 and 5.6%) were reported by Kocak *et al.* (1998); Anuranjini *et al.* (2008); Gücükoğlu *et al.* (2012) and Rahimi (2013), respectively. While, higher results (20, 26, 40, 50, 50, 26, 28 and 23 %) were estimated by Ahmed *et al.* (1988); Masud (1989); Abdel-Hameed and El-Malt (2009); Fadel and Ismail (2009); Zakary *et al.* (2011); Mirzaei *et al.* (2012); Maryam *et al.* (2013) and Abou-Elkhair *et al.* (2014), respectively.

It worth to mention that, the sources of *Staph. aureus* in ice cream may be due to use of infected milk or from infected handlers during preparation and retailing the product.

All the mehallabia samples in this study were negative to *Staph. aureus* isolation (Table 2). The failure of *Staph. aureus* detection in mehallabia samples could be attributed to use of starch during product manufacturing instead of milk or other factors which need further investigation.

Concerning rice with milk, 15% of tested samples gave *Staph. aureus* with counts ranged from  $8 \times 10^4$  to  $8 \times 10^7$  with an average value of  $2.7 \times 10^7$  cells/g. Lower result (4%) was reported by Alisarli *et al.* (2003). Also, *Staph. aureus* was detected in 6.9% of rice cake samples tested by Cho *et al.* (2013).

However, higher results (35 and 66.7%) were estimated by Sina *et al.* (2011) and Tang *et al.* (2015) from rice with sauce and rice products, respectively.

Due to paucity of available literature could be traced dealing with the incidence of *Staph. aureus* in rice with milk at Egypt, therefore, it was hard to discuss the aforementioned result.

Examination of ice cream samples for detection of staphylococcal enterotoxins (SE) revealed that, only one sample (sample No. 19) was positive for enterotoxin type A (Table 3). This result coincided with Rivas *et al.* (1983) as the authors reported that, in ice cream, SEA was the most commonly produced enterotoxin by enterotoxigenic staphylococci. Moreover, several authors (Balaban and Rasooly, 2000; Rall *et al.*, 2008; Imani *et al.*, 2010; Rahimi *et al.*, 2012 and Cho *et al.*, 2013) estimated that, SE type A gene was the predominant gene in enterotoxigenic *Staph. aureus* isolated from various food samples. Furthermore, SE type A was detected in ice cream sample harbouring *Staph. aureus* count exceeded  $10^5$  cfu/ml, while, the counts in the two SEA negative ice cream samples were less than  $10^5$  cfu/ml (Table 3). This observation upheld what obtained by Tranter (1996) who reported that, *Staph. aureus* intoxication occurred when the count exceeded  $10^5$  cfu/g in food.

From the three positive *Staph. aureus* rice with milk samples, two samples (Samples No. 28 and 35) were containing SE type A & C and Type A, respectively (Table 3). It worth to mention that, rice with milk sample No. 28 was containing *Staph. aureus* count of  $8 \times 10^4$  cfu/g and this observation indicated that, even when the staphylococci count less than  $10^5$  cfu/g food, the organism was capable to produce enterotoxins in food.

Rice with milk sample No. 33 was negative for SE, while the count was  $8 \times 10^5$  cfu/g (Table 3). The failure of SE detection in that samples could be attributed to, the expression of enterotoxin genes depends on some factors such as the origin and identity of the bacterial isolate and the host environment of bacteria.

Staphylococcal toxins are fast acting, sometimes causing illness in as little as 30 minutes after eating contaminated foods, but symptoms usually develop within one to six hours. Patients typically experience several of the following: nausea, retching, vomiting, stomach cramps and diarrhea. In more severe cases, dehydration, headache, muscle cramping, and changes in blood pressure and pulse rate may occur (CDC, 2015).

The aforementioned results revealed that, the staphylococcal positive dairy desserts samples

represented the risk of food poisoning to the consumers.

## CONCLUSION

This study revealed that, some dairy desserts sold in Assiut city, Egypt were contaminated with *B. cereus* in a count that sufficient to produce food poisoning. Likewise, *Staph. aureus* enterotoxins were detected in some food samples which representing potential risk of intoxication by consumption of such food. Thorough food inspection and frequent bacteriological surveillance by food control agencies is highly recommended to control the incidence of *B. cereus* and *Staph. aureus* in dairy products to safeguard the consumers from risks of food poisoning.

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### تواجد ميكروبات الباسيليس سيريس والمكور العنقودي الذهبي في بعض الحلويات اللبنية

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تم في هذه الدراسة تجميع عدد ٦٠ عينة من بعض الحلويات اللبنية تشمل الأيس كريم والمهلبية وحلوي الأرز (٢٠ عينة لكل نوع). جُمعت هذه العينات من بعض محلات الألبان بمدينة أسيوط - مصر وقد تم الفحص البكتريولوجي لهذه العينات لعزل وعد كلاً من الباسيليس سيريس والمكور العنقودي الذهبي. وقد كانت نسب عزل الباسيليس سيريس في عينات الأيس كريم والمهلبية وحلوي الأرز هي ٥٥، ٦٠ و ١٥٪ علي التوالي وبمتوسطات عد علي الترتيب هي ٣.١ x ١٠<sup>٦</sup>، ١.٧ x ١٠<sup>٦</sup> و ١.٧ x ١٠<sup>٦</sup> /جم؛ بينما كانت نسبة المكور العنقودي الذهبي هي ١٥٪ لكل من الأيس كريم وحلوي الأرز في حين أعطت عينات المهلبية نتائج سلبية لعزل هذا الميكروب وقد كانت متوسطات عد هذا الميكروب علي الترتيب هي ٦.٧ x ١٠<sup>٦</sup> و ٢.٧ x ١٠<sup>٦</sup> /جم لكلاً من الأيس كريم وحلوي الأرز علي التوالي. وقد تم كشف السموم المعوية A, C لهذا الميكروب في بعض العينات الايجابية للعزل. وأيضاً تمت مناقشة تأثير الميكروبات محل الدراسة علي الصحة العامة.