Zagazig Veterinary Journal
Volume 44, Number 2, p 91-100, September, 2016

DOI: 10.21608/zvjz.2016.7851.

Bacteriological Quality Assessment of Some Locally Manufactured Dairy Desserts Sold in Beni-Suef City, Egypt and Molecular Detection of *Staphylococcus aureus* Enterotoxin Genes

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Article History: Received: 3/8/2016 Received in revised form: 17/8/2016 Accepted: 3/9/2016

Abstract

Seventy-five randomly collected samples of ice-cream, rice with milk and mehallabeia (25, each) from different localities in Beni-Suef city, Egypt, were bacteriologically examined. The mean values of aerobic plate, Coliforms, Fecal coliforms, *E. coli*, Enterococci, *Bacillus cereus* and *Staphylococcus aureus* counts/g in the examined ice-cream samples were $7.02\times10^5\pm4.93\times10^5$, $1.45\times10^5\pm3.21\times10^4$, $9.58\times10^2\pm4.6\times10^2$, $1.35\times10^2\pm66$, $8.12\times10^4\pm4.61\times10^4$, $1.17\times10^4\pm4.59\times10^3$ and $1.51\times10^4\pm9.13\times10^3$, while, in rice with milk samples they were $5.52\times10^4\pm2.78\times10^4$, $3.52\times10^3\pm1.94\times10^3$, $1.66\times10^2\pm1.56\times10^2$,4.8±3.1, $1.28\times10^4\pm6.25\times10^3$, $2.38\times10^5\pm1.4\times10^5$ and $6.32\times10^2\pm2.96\times10^2$, respectively. In mehallabeia samples, the counts were $6.07\times10^4\pm3.6\times10^4$, $6.59\times10^3\pm2.6\times10^3$, $1.6\times10^2\pm89$, 9.48±8, $2.6\times10^4\pm1.64\times10^4$, $3.83\times10^5\pm3.27\times10^5$ and $4.89\times10^2\pm3.28\times10^2$, respectively. Amplification of enterotoxigenic associated genes using multiplex PCR, showed that *sea* and *see* genes were detected in all the examined coagulase positive *S. aureus* isolates. In conclusion, strict hygienic measures should be followed during production and handling of milk products with health educational programs for producers and handlers.

Keywords: Dairy desserts, Bacteriological examination, *sea*, *see*

Introduction

Dairy desserts are popular products worldwide, usually formulated with milk, sugar, modified starch, hydrocolloids such as carrageenan, flavorings and colorants [1,2]. The most widely consumed dairy desserts in Egypt are ice-cream, rice with milk and mehallabeia. Dairy-based foods are important vehicles for the transmission of various pathogens especially in countries where hygienic standards are not strictly enforced [3]. Contaminated milk and its products may harbor a variety of microorganisms which are responsible for many food-borne outbreaks such as Coliforms, Fecal coliforms, *E. coli*, Enterococci, *Bacillus cereus* and *Staphylococcus aureus* [4-8].

Staphylococcus aureus is considered the second or third most common pathogen responsible for outbreaks of food poisoning in many countries [9]. Strains of *S. aureus* can produce one or more staphylococcal enterotoxins (SEs), which cause food poisoning in humans [10]. Up to date, 21 staphylococcal enterotoxins (SE) or enterotoxin-like proteins (SEls) have been identified [11]. On the basis of antigenic characters, Staphylococcal enterotoxins are classified into five main serological types characterized by the initials SEA, SEB, SEC, SED and SEE [11,12].

The common feature of SEs is high stability and resistance towards most proteolytic enzymes, such as pepsin or trypsin, allowing protection of their activity in the gastrointestinal tract [13]. The SEs also retain their biological activities even after pasteurization [14]. The objectives of this study were to evaluate the bacteriological quality of some dairy desserts (ice-cream, rice with milk and mehallabeia) consumed in Beni-

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Suef city, Egypt as well as the molecular detection of some enterotoxigenic associated genes in *S. aureus* isolates using multiplex PCR. to verify the quality of these products.

Material and Methods

Collection and preparation of samples

Seventy-five samples of dairy desserts including ice-cream, rice with milk and mehallabeia (25, each) were randomly collected from different localities at Beni-Suef city, Egypt. The collected samples were directly transferred aseptically in sterile bags to the laboratory with a minimum of delay to be examined.

Preparation of samples

Eleven grams of each sample were thoroughly mixed and transferred to a sterile wide-mouth container containing 99 mL of sterile saline and they were mixed until a homogenous dispersion was obtained to reach a dilution of 1:10, from which ten-fold serial dilutions were prepared [15].

Bacteriological examination

The bacteriological quality of the prepared samples was evaluated by the determination of the aerobic total colony count using standard plate count agar at 35°C for 48 ± 3 h, also, the total coliform count by Most Probable Number method using Lauryl Sulphate Tryptose broth and Brilliant-green Lactose Bile broth 2% at 35±1ºC for 24 h was determined [15]. Fecal coliform count by Modified Eijkman's test using EC broth at 45.5ºC ± 0.2 for 24 ± 2 h was also carried out [16]. *Escherichia coli* true fecal type was isolated and identified using Eosine Methylene Blue agar (EMB) at 35ºC for 24 h were carried out according to AOAC [17]. Enterococci count using Kanamycin Aesculin Azide agar at 42 ºC for 18-24 h [18] and enumeration and isolation of *B. cereus* using Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue agar (PEMBA) which incubated at 37 ºC for 24 h [16,18]. Moreover, enumeration and isolation of coagulase positive *S. aureus* using Baired Parker's agar at 37ºC for 24 h [19,20].

Detection of enterotoxigenic genes in S. aureus using multiplex PCR

Three coagulase positive *S. aureus* isolates recovered from ice-cream, rice with milk and mehallabeia (one isolate, each) were used for the detection of enterotoxigenic associated genes using multiplex PCR [21].

Genomic DNA from three coagulase positive *S. aureus* isolates was extracted using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer guidelines. Specific primers for the amplification of the five major enterotoxin associated genes were synthesized by Metabion Company, Germany, and the sequences are listed in Table (1).

The reaction was performed in a total volume of 50 µl containing 25 µL of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µL of each primer (20 pmol), 5 µl of molecular water, and 10 µL of template DNA. The reaction conditions included initial denaturation (94ºC for 5 min), 35 cycles (denaturation at 94ºC for 30 sec; annealing at 50ºC for 45 sec; extension at 72ºC for 45 sec) and final extension at 72°C for 10 minutes [21].

Ten µL of the amplified PCR product was analyzed by electrophoresis in 1.5% agarose gel (Applichem, Germany, GmbH) stained with 0.5 µg of ethidium bromide/mL (Sigma). Electrophoresis was carried out in 1X TAE buffer using gradients of 5V/cm for 1 hour. DNA molecular size marker (100 bp ladder) (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was visualized and photographed by a gel documentation system (Alpha Innotech, Biometra).

Table (1): Oligonucleotide sequences of primers for the amplification of five enterotoxin associated genes in *S. aureus* isolates

Target gene	Primers sequences (5'-3')	Amplified segment (bp)
sea	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102
seb	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164
sec	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451
sed	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278
see	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209

Results and Discussion

The results recorded in Table (2) showed that the aerobic plate counts (APC) ranged from $1x10^2$ to $1.1x10^7$ with a mean value of $7.02\times10^5\pm4.93\times10^5$ in the examined ice-cream samples. While, in the rice with milk samples APC ranged from $1x10^2$ to $6x10^5$ with a mean value of $5.52\times10^4\pm2.78\times10^4$ (Table 3). In mehallabeia samples, APC ranged from $2.3x10^2$ to $9x10^5$ with a mean value of $6.07\times10^4\pm3.6\times10^4$ (Table 4). Similar results were recorded by Abdel-Haleem *et al*. [22] and Al-Gendi [23]. Higher results for ice-cream were recorded by El-Bagoury [24] and Sobeih *et al*. [25].

The high aerobic plate counts reflect the inferior quality of raw materials and ingredients used in manufacturing of these products, insufficient heat-treatment, post heat-treatment contamination as well as unhygienic conditions during production.

Table 2: Statistical analytical results of bacterial counts in examined ice-cream samples

Count	Number of samples	Positive samples		Counts / g		
		No.	%	minimum	maximum	Mean ± SEM
APC	25	25	100	$1x10^2$	$1.1x10^7$	$7.02\times10^5\pm4.93\times10^5$
Coliforms	25	23	92	<3	$4.3x10^5$	$1.45\times10^5\pm3.21\times10^4$
Coliforms	25	15	60	<3	$9x10^3$	$9.58\times10^2\pm4.6\times10^2$
E. coli	25	15	60	<3	$1.5x10^3$	$1.35\times10^2\pm66$
Enterococci	25	16	64	<100	$1.1x10^6$	$8.12\times10^4\pm4.61\times10^4$
B. cereus	25	10	40	<100	$7.3x10^4$	$1.17\times10^4\pm4.59\times10^3$
S. aureus	25	13	52	<100	$2x10^5$	$1.51\times10^4\pm9.13\times10^3$

APC: Aerobic Plate Count

The examination of dairy products for coliforms is highly recommended for their significance as indicator organisms for the unhygienic measures adopted in dairy industries. Moreover, the high number of coliforms is objectionable as it renders these products to be considered of an inferior quality. Regarding the results recorded in Table (2), coliforms could be detected in 92% of the examined ice-cream samples with counts ranged from <3 to $4.3x10^5$ with a mean value of $1.45\times10^5\pm3.21\times10^4$, while, 48% of rice with milk samples ranged from <3 to $4x10^4$ with a mean value of $3.52\times10^3\pm1.94\times10^3$ (Table 3) and also in 52% mehallabeia samples it ranged from <3 to 4.33×10^4 with a mean value of $6.59\times10^3\pm2.6\times10^3$ (Table 4). Nearly similar results for ice-cream and rice with milk were recorded by Al-Gendi [23] who also reported

higher rates for mehallabeia. Lower isolation rates and counts for rice with milk and mehallabeia were reported by Abdel-Haleem *et al*. [22] who detected higher results for ice-cream. Moreover, lower isolation rates and higher counts for ice-cream were recorded by El-Bagoury [24]. The presence of high number of Coliforms in the examined samples is considered to be an efficient indicator for fecal contamination and therefore, the possibility of presence of hazardous organisms. Moreover, it can indicate the use of poor quality ingredients and /or the absence of heat-treatment and sanitization during processing. Even when good hygienic measures are adopted during the manufacture of these dairy products, contamination might occur during the packaging of the finished product or even due to careless handling, storage and distribution [16].

Table 3: Statistical analytical results of bacterial counts in examined rice with milk samples

Count	Number of samples	Positive samples		Counts / g		
		No.	%	minimum	maximum	Mean ± SEM
APC	25	25	100	$1x10^2$	$6x10^5$	$5.52\times10^4\pm2.78\times10^4$
Coliforms	25	12	48	<3	$4x10^4$	$3.52\times10^3\pm1.94\times10^3$
Coliforms	25	4	16	<3	$3.9x10^3$	$1.66\times10^2\pm1.56\times10^2$
E. coli	25	4	16	<3	70	4.8±3.1
Enterococci	25	10	40	<100	$1.1x10^5$	$1.28\times10^4\pm6.25\times10^3$
B. cereus	25	12	48	<100	$2.6x10^6$	$2.38\times10^5\pm1.4\times10^5$
S. aureus	25	11	44	<100	$5.6x10^3$	$6.32\times10^2\pm2.96\times10^2$

APC: Aerobic Plate Count

Fecal coliforms could be detected in 60% of the examined ice-cream samples with counts ranged from <3 to $9x10^3$ with a mean value of $9.58\times10^2\pm4.6\times10^2$, while, 16% of the examined rice with milk samples contained coliforms with counts ranged from <3 to $3.9x10^3$ with a mean value of 1.66×10^2 $\pm1.56\times10^2$ (Tables 2 and 3). In 24% of mehallabeia samples, fecal coliforms count ranged from <3to $2.1x10^3$ with a mean value of $1.6\times10^2\pm89$ (Table 4). Relatively similar findings were recorded by Abdel-Haleem *et al*. [22] for ice-cream and mehallabeia who cited higher results for rice with milk, also, higher results recorded by Al-Gendi [23] for ice-cream, rice with milk and mehallabeia.

The presence of fecal coliforms in a dairy product indicates either direct or indirect fecal contamination because it is a common inhabitant in the intestinal tract of humans and animals. Moreover, there is a close link between *E. coli* and possibly enteric pathogens. Also, the presence of fecal coliforms may be attributed to poor quality ingredients, ineffective sanitization, unhygienic storage and carelessness during handling and distribution [16].

Table 4: Statistical analytical results of bacterial counts in examined mehallabeia samples

Count	Number of samples	Positive samples		Counts / g		
		No.	%	minimum	maximum	Mean ± SEM
APC	25	25	100	$2.3x10^2$	$9x10^5$	$6.07\times10^4\pm3.6\times10^4$
Coliforms	25	13	52	<3	$4.33x10^4$	$6.59\times10^3\pm2.6\times10^3$
Coliforms	25	6	24	<3	$2.1x10^3$	$1.6\times10^2\pm89$
E. coli	25	6	24	<3	$2x10^2$	9.48±8
Enterococci	25	12	48	<100	$4x10^5$	$2.6\times10^4\pm1.64\times10^4$
B. cereus	25	12	48	<100	$8.2x10^6$	$3.83\times10^5\pm3.27\times10^5$
S. aureus	25	4	16	<100	$7x10^3$	$4.89\times10^2\pm3.28\times10^2$

APC: Aerobic Plate Count

As illustrated in Table (2) *E. coli* was detected in 60% of ice-cream samples with counts ranged from <3 to $1.5x10^3$ with a mean value of 1. 35×102±66.While, in 16% of rice with milk samples *E. coli* count ranged from <3 to 70 with a mean value of 4.8±3.1(Table 3). In 24% of mehallabeia samples it ranged from <3 to $2×10^2$ with a mean value of 9.48±8 (Table 4).

Abdel Haleem *et al*. [22] isolated *E. coli* from 40%, 24% and 20% with counts $<10^4$, $<10^2$ and <10 in ice cream, rice with milk and mehallabeia samples, respectively. Al-Gendi [23] isolated *E. coli* from 20%, 0% and 4% with counts $<10^4$, <3 and $<10^2$ in ice cream, rice with milk and mehallabeia samples, respectively. In addition, Mohammed *et al*. [26] isolated *E. coli* from 13.3% of ice cream samples.

The results given in Table (2) showed that enterococci were detected in 64% of ice-cream samples with count ranged from <100 to $1.1x10^6$ with a mean value of $8.12×10^4$ $±4.61×10^4$. While, 40% rice with milk samples contained enterococci count ranged from <100 to $1.1×10^5$ with a mean value of $1.28×10^4$ $±6.25×10^3$ (Table 3). In 48% of mehallabeia samples it ranged from <100 to $4x10^5$ with a mean value of $2.6×10^4±1.64×10^4$ (Table 4).

Abdel Haleem *et al*. [22] reported that 53.3% of the examined mehallabeia samples were contaminated by enterococci with a count less than 100/g and in 48% of rice with milk samples with a count below 100/g. In addition, 86.6% of ice-cream samples were contaminated by enterococci with a count varied from 10 to $6.4x10^4$ with a mean count of $8.1x10^3$ [22].

The obtained high values of enterococci reflect the poor sanitary practices during manufacturing, handling, storage and distribution as its occurrence is considered as an indicator of fecal matter contamination. Also, these high numbers could constitute a public health hazard and may induce food poisoning [16].

The results recorded in Tables (2 and 3) showed that *B. cereus* was detected in 40% of ice-cream samples with a range of <100 to $7.3x10^4$ with a mean count of $1.17×10^4$ $±4.59×10^3$, while in48% of rice with milk samples it ranged from <100 to $2.6×10^6$ with a mean value of $2.38×10^5±1.4×10^5$. In addition, 48% of mehallabeia samples, *B. cereus* count ranged from <100 to $8.2×10^6$ with a mean value of $3.83×10^5±3.27×10^5$ (Table 4).

Hussien *et al*. [27] isolated *B. cereus* form 55% of ice-cream samples with a count ranging from $2x10^6$ to $3x10^{10}$ with a mean count of $3.1x10^9$. Lower results (16 and 4%) were recorded by Altaf *et al*. [28] and Maryam *et al*. [29], respectively. The high percentage of *B. cereus* 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 [27].

Similar results were reported by AL-Ashmawy *et al*. [30] for mehallabeia while higher results were recorded by Hussien *et al*. [27].

Examining foods for *S. aureus* aims to confirm that these organisms may be the causative agent of foodborne illness, to determine whether the product or its ingredients are the potential source of *S. aureus* food poisoning and to demonstrate post-processing contamination which mainly due to defect in the personal hygiene or exposure of the food to inadequately sanitized food processing surfaces [16].

The results recorded in Table (2) showed that *S. aureus* was detected in 52% of ice-cream samples with a range of <100 to $2x10^5$ with a mean count of $1.51×10^4±9.13×10^3$. In 44% of rice with milk samples the count ranged from <100 to $5.6x10^3$ with a mean value of $6.32×10^2±2.96×10^2$ (Table 3). While, in 16% of mehallabeia samples it ranged from <100 to $7x10^3$ with a mean value of $4.89×10^2±3.28×10^2$ (Table 4).

Regarding ice-cream, the results recorded by Hussein *et al*. [27] showed the isolation of *S. aureus* from 15% of the samples with a count ranged from $1{,}9x10^3$ to $2x10^6$with a mean count of $6.7x10^5$. Mathews *et al*. [31] isolated *S. aureus* from 18.7% of ice-cream samples. Similar results (40%, 50%and50%) were recorded by Abdel Hameed and EL-Malt [32], Fadel and Ismail [33] and Zakary *et al*. [34], respectively. However, higher results were recorded by El Bagoury [24] and Sobeih et al. [25] respectively. In addition, Hussein *et al*. [27] couldn't detect *S. aureus* in mehallabeia samples.

Similar results for rice with milk (35 and 66.7%) were recorded by Sina *et al*. [35] and Tang *et al*. [36], respectively, while, lower results (6.9 and 15%) were recorded by Cho *et al*. [37] and Hussein *et al*. [27], respectively.

Egyptian standards (1993) recommended that ice cream must be free from *S. aureus*, while WHO [39] stated that *S. aureus* should not increase than 10 CFU/g. Also IDF [40] stated that the number of *S. aureus* in frozen milk based products should not exceed 100 CFU/g. *S. aureus* is the leading cause of foodborne intoxication (minimum infection dose 10^5-10^7CFU/g or 1-20µg enterotoxin per person) [41].

In the current study, three coagulase positive *S. aureus* isolates recovered from ice cream, rice with milk and mehallabeia (one, each) were used in order to detect the classical enterotoxin associated genes (*sea*, *seb*, *sec*, *sed* and *see*) using multiplex PCR. The results demonstrated that, *sea* and *see* genes were found in the three investigated isolates (Figure 1).

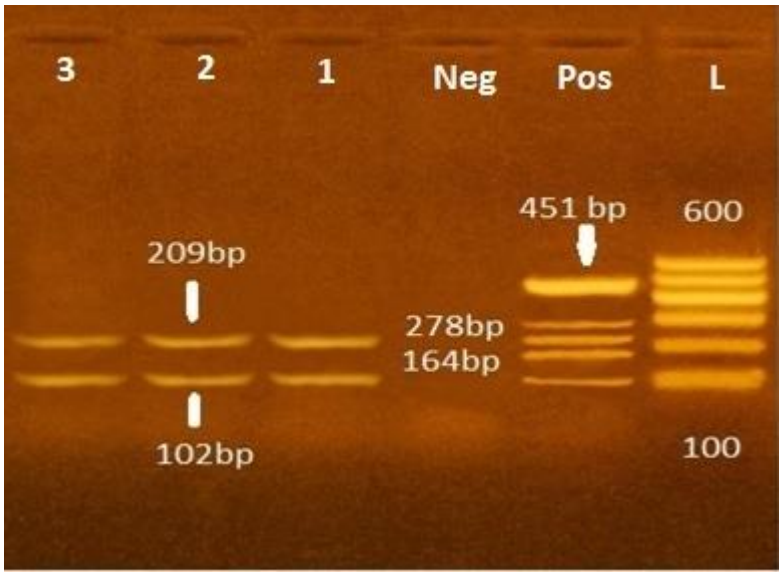

Figure 1: Agarose gel electrophoresis patterns of multiplex PCR amplification products for *S. aureus* enterotoxin associated genes. Lane L, DNA molecular size marker (100 bp ladder), Lane Pos., control positive mixed strains for classical enterotoxins in *S. aureus* A, B, C, D and E prepared by the National Laboratory for Veterinary Quality Control on Poultry, Dokki, Giza, Lane Neg., control negative strain for classical enterotoxins in *S. aureus* (A, B, C, D and E), lanes 1 to 3, PCR amplicons for three coagulase positive *S. aureus* isolates recovered from ice-cream, rice with milk and mehallabeia samples. The three isolates had both *sea* and *see* enterotoxin associated genes at 102 bp and 209 bp, respectively.

Recent studies have reported that most *S. aureus* strains isolated from milk and dairy products harbored more than one toxin gene [42,43]. Similar findings were reported by Mathenge *et al*. [9] who concluded that out of the screened 270 *S. aureus* isolates, *sea* (61.8%) was the most frequently detected gene, followed by *see* (33.1%). Madahi *et al*. [44] reported that the most commonly detected gene in 27 *S. aureus* isolates was *sea* (25%),

but no *see* gene was detected. The differences in the frequency of enterotoxin associated genes in *S. aureus* might be attributed to the origin of the isolates, which could vary between animals, humans, foods or environment [9]. The role of SEs in staphylococcal food poisoning is still not clear in most parts of the world; further studies are needed to assess the role they play in *S. aureus* food-poisoning.

Conclusion

From this study it is concluded that sanitary measures during manufacturing, handling and distribution of milk based desserts used for human consumption in Beni-Suef city are neglected. Most examined samples are highly contaminated rendering them of inferior quality and unfit for human consumption exposing the consumers to health hazards. Therefore, strict hygienic measures should be followed during production and handling of these products with health educational programs for producers and handlers.

Conflict of interest

The author declares no conflict of interest.

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الملخص العربي

تقييم الجودة البكتيريولوجية لبعض الحلوى اللبنية المصنعة محليا و المباعة فى مدينة بنى سويف مع تحديد السموم المعوية للمكور العنقودى الذهبى باستخدام سلسلة تفاعلات انزيم البلمرة المتعددة

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تضمن البحث فحص ٧٥ عينة من الايس كريم والارز باللبن و المهلبية (٢٥ عينة من كل منتج) جمعت عشوائيا من اماكن مختلفة فى مدينة بنى سويف. وقد اظهرت النتائج التلوث الكبير للعينات حيث تواجدت الميكروبات الهوائية والقولونية والقولونية البرازية وميكروب الايشريشيا كولا وكذلك مجموعة االمكورات السبحية المعوية وميكروب الباسيلس سيريس و الميكروب المكور العنقودى الذهبى بمتوسطات أعداد٧.٠٢× ١٠[٥] ± ٤.٩٣ × ١٠[٥] و ١.٤٥× ١٠[٥] ± ٣.٢١ × ١٠[٤] و٩.٥٨× ١٠[٢] ± ٤.٦ × ١٠[٢] و١.٣٥× ١٠[٢] ± ٦٦ و٨.١٢× ١٠[٤] ± ٤.٦١ × ١٠[٤] و١.١٧× ١٠[٤] ± ٤.٥٩ × ١٠[٣] و١.٥١× ١٠[٤] ± ٩.١٣ × ١٠[٣] فى الايس كريم بينما كانت فى عينات الارز باللبن ٥.٥٢× ١٠[٤] ± ٢.٧٨ × ١٠[٤] و ٣.٥٢× ١٠[٣] ± ١.٩٤ × ١٠[٣] و١.٦٦× ١٠[٢] ± ١.٥٦ × ١٠[٢] و٤.٨ ± ٣.١ و١.٢٨× ١٠[٤] ± ٦.٢٥ × ١٠[٣] و٢.٣٨× ١٠[٥] ± ١.٤ × ١٠[٥] و٦.٣٢× ١٠[٢] ± ٢.٩٦ × ١٠[٢] اما فى عينات المهلبية فقد كانت موجودة بمتوسطات ٦.٠٧× ١٠[٤] ± ٣.٦ × ١٠[٤] و ٦.٥٩× ١٠[٣] ± ٢.٦ × ١٠[٣] و١.٦× ١٠[٢] ± ٨٩ و٩.٤٨ ± ٨ و٢.٦× ١٠[٤] ± ١.٦٤ × ١٠[٤] و٣.٨٣× ١٠[٥] ± ٣.٢٧ × ١٠[٥] و٤.٨٩× ١٠[٢] ± ٣.٢٨ × ١٠[٢] على الترتيب وقد تم الكشف عن جينات السموم المعوية لبعض العترات المعزولة من ميكروب المكور العنقودى الذهبى باستخدام سلسلة تفاعلات انزيم البلمرة المتعدد حيث وجد كل من جينى sea و see فى العترات المختبرة. من نتائج هذه الدراسة انه ينبغي اتباع التدابير الصحية الصارمة أثناء الإنتاج والتعامل مع هذه المنتجات مع ضرورة عمل برامج تعليمية إرشادية للمنتجين والمتعاملون مع الأغذية.