Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 50(4) (2012), 417– 426 **http://annagricmoshj.com**

Detection of *Staphylococcus aureus* enterotoxins in milk, milk products as well as milk handlers in Sharkia Governorate

Elsayed M. Abd El-Wahed
Food Science Department, Faculty of Agriculture, Zagazig University, Egypt

Abstract

Staphylococus aureus may contain one or more genes that encode staphylococcal enterotoxins (SE) that cause food poisoning. The previously known toxins were the five major classical types; however, with the extensive analysis of the *Staph. aureus* genome, new genes encoding enterotoxin-like superantigens have been identified. Milk and dairy products are frequently contaminated with enterotoxigenic *Staph. aureus,* which is often involved in staphylococcal food poisoning; these contaminations are either from animal or human sources.

This work aimed to detect types of enterotoxins produced by *Staph. aureus* isolated from milk, kariesh cheese and ice-cream samples and from nasal swabs got from food handlers, the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and PCR was used.

In this work, 450 samples of Milk, ice-cream, kariesh cheese and nasal swabs from food handlers were examined for the presence of Coagulase positive *Staph. aureus*, using Mannitol salt agar, Baird-Parker agar, tube coagulase test, and latex agglutination test for protein A and capsular polysaccharides. Confirmed *Staph. aureus* isolates were examined for the production of SEs using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and the type of SE genes by polymerase chain reaction (PCR).

The Coagulase positive *Staph. aureus* isolates were detected in 84% of Staph colonized raw milk, 87.5% of Staph colonized ice-cream and 92.8% of Staph colonized kariesh cheese samples and 50% of Staph colonized nasal swabs, with 62.5% of total staph colonization which exceeds the Egyptian Standards. Collectively, 49.5% of coagulase positive *Staph. aureus* isolates were enterotoxigenic and the highest percentages were detected in raw milk taken directly from animals (68.7%) and kariesh cheese from street distributors (65.7%). In all samples, the major classical enterotoxin genotype was SEA which was detected in 33 isolates of toxigenic isolates. SEC was detected in 17 isolates and SED in 11 isolates. SEB could not be detected. For the newly described genes, SEG was detected in 11 isolates and SEH in 8 isolates. Mixed forms were found in 25 isolated of toxigenic isolates and four strains carried undescribed genes.

Therefore we concluded that: Raw milk and some dairy products in the markets in Sharkia Governorate, Egypt are contaminated with enterotoxigenic *Staph. aureus.* The most common type in both milk and dairy products as well as in nasal swabs was SEA which is known to be less common among strains from animal origin than from human. Nasal carriage in human food handlers is considered a primary source of contamination of milk and dairy products.

Key words: *Staphylococcus aureus*, enterotoxins, milk products, Sharkia Governorate

Introduction

Staphylococcus aureus is a common pathogen that colonizes and produces disease in a variety of hosts (**Jarraud *et al.*, 2002**). *Staph. aureus* may contain one or more genes that encode a variety of immunomodulatory pyrogenic toxins (PTs), including the staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST). The PTs interact with several cellular targets to produce disease, such as food poisoning and toxic shock syndrome (**Omoe *et al.*, 2002**).

Staph Food Poisoning (SFP) is a mild intoxication occurring after the ingestion of food containing staphylococcal enterotoxins (SEs) (**Chiang *et al.*, 2008**). The only previously known toxins were the five major classical types, i.e, SEA, SEB, SEC, SED, and SEE. However, with the extensive analysis of the *Staph. aureus* genome allowing the identification of new genes encoding enterotoxin-like superantigens (SELs) such as SEG to SEU. Some of these are thought to be involved in staphylococcal food poisoning (**Rall *et al.*, 2008**).

Staph. aureus is often responsible for intramammary infection in bovines, and is the main aetiological agent of contagious clinical/sub-clinical mastitis in dairy herds (**Taverna *et al.*, 2007**).

The organism may colonize the teat skin and teat canal, which may predispose to intramammary infection leading to mastitis. The bacterium adheres to the internal mucosal surfaces, producing several virulence factors (**Leloir *et al.*, 2003**). Milk and dairy products are frequently contaminated with enterotoxigenic *Staph. aureus*, which is often involved in staphylococcal food poisoning; these contaminations are either from animal or human sources. Recently by the identification of the new SEs, the perceived frequency of enterotoxigenic strains has increased, suggesting that the pathogenic potential of Staphylococci may be higher than previously thought (**Zschoch *et al.*, 2005**).

As milk is a very suitable medium for the growth of many pathogens including *Staph. aureus* and because raw milk is subjected to contamination either directly or indirectly from different sources including producing animal, milk producers and handlers, many outbreaks of SFP traceable to dairy products do still occur inspite of advanced dairy manufacturing processes (**Srinivasan *et al.*, 2006**). Nasal carriage is considered a primary source of contamination of manually handled food as well as a source of *Staph. aureus* transmission between human. Approximately 20% of healthy humans are estimated to be persistently colonized by *Staph. aureus*, while as many as 60% can be colonized intermittently (**Bania *et al.*, 2006**). It was demonstrated that *Staph. aureus* is associated with some human diseases as well as that *Staph. aureus* isolated from healthy carriers share a very high prevalence of enterotoxin gene cluster (**Mempel *et al.*, 2008**).

The throat and nasal earners of pathogenic *Staph. aureus* in normal community of healthy individuals give opportunities for post-pasteurization contamination. So, further studies should be done to clarify the epidemiological association and bacteriological characteristics of human and animal S. aureus in food (**Hata *et al.*, 2008**).

Aim of the work

To detect the presence and prevalence of coagulase positive *Staph. aureus* in milk, kariesh cheese and ice-cream samples and in nasal swabs from food handlers, and to detect *Staph. aureus* enterotoxins by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and to detect the type of enterotoxins genes by PCR.

Materials and methods

This study is a cross section study that was conducted in Sharkia Governorate from January 2012 till September 2012 on 450 samples (200 milk samples, 100 ice-cream, 100 kariesh cheese and 50 nasal swabs from food handlers. The nasal swab samples were conducted by Doctor W.F. Elkhateeb, Faculty of Pharmacy, Ain Shams University.

Samples preparation:

Milk samples: 10 ml added to 90 ml of sterile saline, then ten fold serial dilutions were carried out. Ice-cream samples: were cooled to 2°C in an ice box and were left in water bath at 44°C for not more than 15 minutes to melt, then they were thoroughly mixed by a sterile stirrer. 10 ml were transferred to 90 ml of sterile saline, and then ten fold serial dilutions were carried out. kariesh cheese samples: Ten gram from each sample were mashed thoroughly in a sterile mortar and 90 ml of 2% sodium citrate were added to obtain a dilution of 1:10, then ten fold serial dilutions were carried out. Nasal samples from food handlers that deal with milk and dairy products (**A.P.H.A. 1992**).

Isolation, identification and confirmation of *S. aureus*:

10 ml or gm of the prepared samples and nasal swabs were inoculated into 10 ml of Staphylococcus broth (Difco) and incubated at 35°C for 20 h (**FAO 1992**). Each inoculum was subculture on selective media as Mannitol Salt agar (Bio Merieux, BBL, 11407) (**Finegold and Martin, 1994**). Colonies of *Staph. aureus* were identified by lecithinase and lipase activities on Baird-Parker agar (Oxoid, BO0458J, Basingstoke, England) (**Finegold and Martin, 1994**). Then, suspected *Staph. aureus* isolates were confirmed by tube coagulase test (**Macfadin, 1980**) and detection of clumping factor, protein A and capsular polysaccharides by latex agglutination test using Oxoid Dry Spot Staphytect Plus (DR 100 M) which uses blue latex particles coated with both porcine fibrinogen and rabbit immunoglobulin G (IgG) including specific antibodies raised against capsular polysaccharides of *Staph. aureus* (**Wanger *et al.*, 1992**).

Viable count of *S. aureus* in milk and dairy products;

A quantity of 0.1ml from each prepared dilutions of samples was cultured on BP-A, using surface plating technique (**Thatcher and Clark, 1975**). The number of suspected colonies in countable plates was enumerated and the *Staph. aureus* count per ml or gm was calculated and recorded.

Detection of enterotoxigenicity by SDS-PAGE (Laemmli, 1970):

-Samples preparation:

One colony of each strain was washed and stirred after the addition of 25 µl SDS lysing buffer, and the proteins were denatured in boiling water for 5 min. Supernatant was then centrifuged and collected in an eppendorf tubes for electrophoresis.

-Preparation of Polyacrylamide Gels:

Two parts of discontinuous gels were used; the stacking gel (the upper portion) has a low acrylamide concentration (4.5%), low pH (6.8) and low resolving ability. The resolving gel (the lower portion) has 12.5% acrylamide concentration, much smaller pores and pH 8.8.

-Sample Application and Electrophoresis:

10 µl of the protein extracts of tested strains with Page Ruler prestained protein ladder (SM0671 Fermentas Life Sciences) that was used as a protein marker. The gel was electrophoresed by using Hoefer Mighty Mini-Vertical Unit with bromo phenol blue dye for 4 hrs. ±10 min. until the dye migrates 100mm down the length of separating gel.

-Protein Visualization:

After electrophoresis, the gels were fixed and stained with 1% Coomassie Blue R-250 with gentle agitation overnight (16 h.). The stain solution was

removed and replaced with ethanol and acetic acid. The separated proteins of SEs ranged from 25-30 kDa and were visualized by Ultraviolet transilluminator Hoefer Scientific Instrument (Cat No. 7015668).

Detection of SEs genes by polymerase chain reaction (PCR) (Bendahou *et al.*, 2009):

1-Extraction of *Staph. aureus* DNA:

Manual extraction from fresh culture by heating at 90°C for 17 minutes, then keeping at -20°C until needed. When required, 10 µl of the supernatant of thawed and centrifuged sample was used.

2-Amplification of SEs genes:

PCR protocol was performed for individual gene, as a series of separate reactions, using only one primer pair for each reaction mixture. PCR amplification was conducted in final volume of 25 µl. The PCR mixture consisted of 3 µl of the forward primer, 3 µl of the reverse primer (Metabion, Germany) and 10 µl of the extracted DNA. The mixture was added to 9 µl of Master Mix which consisted of dd H_2O, 2.5 mM concentration of each deoxynucleoside triphosphate (dNTP) (Jena Bioscience, Cat No. NU-1005S), 2U of BioReady r Taq DNA polymerase (BioFlux- Cat No. BSA12M1) and 10 x PCR buffer with 15 mM $MgCl_2$ (Jena Bioscience).The primers used for detection of SE genes are listed in table (1). A PERKIN ELMER Gene Amp 9600 PCR System was used for amplification. The thermal cycling conditions were as follow: initial cycle of denaturation (at 95°C for 5 min), followed by 30 cycles of denaturation at (95 °C for 15 s), annealing (at 50°C for 30 s), and extension (at 72 °C for 30 s), with a final extension step of 72°C for 8 min.

3-Detection of amplified products:

Ten µl of PCR products were mixed with 2 µl of 6x loading dye (Jena Bioscience) and were added to the wells of 1.5% agarose gel (Molecular Biology Grade) stained with ethedium bromide (0.5 µl /ml) and electrophoresed in 5x TBE buffer (Tris-Boric-EDTA) at 100 V for 30 min. PCR products were sized against a 100 bp DNA step ladder (5 µl of ladder was added in one well) (Cat No. 239125, Qiagen, Germany) and were visualized in the gel using U.V. transilluminator (Hoefer Scientific).

Table 1. The Primers used for detection of SEs genes (Astrid *et al.*, 2004)

Primer	Primer Sequence	Product Size (bp)
SEA forw.	GCAGGGAACAGCTTTAGGC	521
SEA rev.	GTTCTGTAGAAGTATGAAACACG	
SEB forw	ACATGTAATTTTGATATTCGCACTG	667
SEB rev.	TGCAGGCATCATGTCATACCA	
SEC forw.	CTTGTATGTATGGAGGAATAACAA	284
SEC rev.	TGCAGGCATATCATACCA	
SED forw.	GTGGTGAAATAGATAGGACTGC	385
SED rev.	ATATGAAGGTGCTCTGTGG	
SEG forw.	CGTCTCCACCTGTTGAAGG	328
SEGrev.	CCAAGTGATTGTCTATTGTCG	
SEH forw.	CAACTGCTGATTTAGCTCAG	359
SEHrev	GTCGAATGAGTAATCTCTAGG	

Statistical analysis:

Was performed using SPSS software version 16 (SPSS Inc., Chicago, USA), and expressed as mean, standard deviation (SD), number and percentage. Statistical methods were applied including descriptive statistics (frequency, percentage, mean and SD) and tests of significance (X2 and Fisher exact tests for categorical variables and Student T-test and Mann Whitney tests for continuous variables). Statistical significance was assumed when $P < 0.05$.

Results and Discussion

This study was conducted on 450 different samples which were classified as 200 raw milk samples, 100 ice-cream samples, 100 kariesh cheese samples and 50 nasal swabs from food handlers.

Table 2: shows that 59.5%, 40%, and 70% of raw milk, ice cream, and Kariesh cheese samples respectively were colonized by Staphylococcus species organisms with 48.7%, 100%, and 100% of them respectively were exceeding Egyptian Standards and 80% of nasal samples were colonized by Staphylococcus species. Figure (1) shows the colony count of *Staph. aureus* organisms in different types of samples.

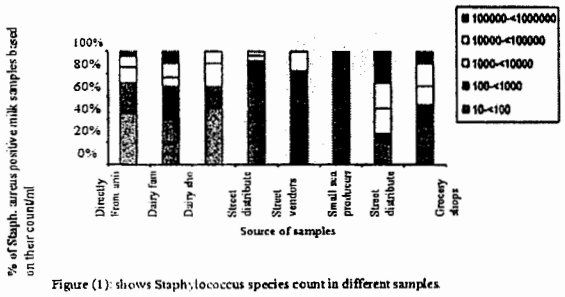

Figure (1): shows Staphylococcus species count in different samples

Table 2. Prevalence of Staph. colonization and their count in different samples

Type of sample	No of samples	%of positive samples	Count/ml (cfu)	Exceeding E.S	CPS	CNS
Raw milk samples						
From animals	50	22 (44%)	10-<10^2- 2x10^5	12 (54.5%)	16 (72.7%)	6 (27.3%)
Dairy farms	50	40 (80%)	10-<10^2- 2.8×10^5	24 (60%)	35 (87.5%)	5 (12.5%)
Dairy shops	50	30 (60%)	10-<10^2- 2×10^4	15 (50%)	25 (83.3%)	5 (16.7%)
Street distributors	50	27 (54%)	10-<10^2- 1.7×10^4	7 (26%)	24 (88.9%)	3 (11.1%)
P- value		<0.03	< 0.001	0.253	0.3	0.4
Total	200	119 (59.5%)	10-<10^2- 2.8×10^5	58 (48.7%)	100 (84%)	19 (16%)
Ice- cream samples						
Street vendors	50	25 (50%)	10-<10^2- 4×10^3	25 (100%)	22 (88%)	3 (12%)
Small scale producers	50	15 (30%)	10-<10^2- 2×10^2	15 (100%)	13 (86.7%)	2 (13.3%)
P- value		0.351	<0.03		0.642	0.591
Total	100	40 (40%)	10-<10^2 - 4×10^3	40 (100%)	35 (87.5)	5 (12.5%)
Kariesh cheese samples						
Street distributors	50	40 (80%)	10-<10^2-5×10^5	40 (80%)	38 (95%)	2 (5%)
Grocery shops	50	30 (60%)	10-<10^2 -2×10^5	30 (60%)	27 (90%)	3 (10%)
P- value		0.592	0.475	0.628	0.624	0.483
Total	100	70 (70%)	10-<10^2 -5×10^5	70 (100%)	65 (92.8%)	5 (7.2%)
Nasal swabs						
Nasal swabs	50	40 (80%)	-	-	20 (50%)	20 (50%)
P- value		<0.03		-	<0.04	<0.01
Total	450	269 (59.8%)	-	168 (62.5%)	220 (81.8%)	49 (18.2%)
P- value for total samples		P<0.02*	P0.001**	P<0.000***	P<0.03*	P<0.01*

NB.: cfu: colony forming unit; ES: Egyptian standards, CPS: Coagulase Positive Staphylococci, CNS: Coagulase Negative Staphylococci.

Table (3) shows that 49%, 42.8%, 53.8% and 50% of raw milk, ice cream, Kariesh cheese and nasal samples respectively, were colonized by enterotoxigenic Coagulase Positive Staphylococci.

Table (4) shows that 33%, 17%, 11%, 11% and 8% of enterotoxigenic strains were encoded by SEA, SEC, SED, SEG and SEH genes respectively. But SEB gene was not detected in any enterotoxigenic isolated strains.

Table (5) shows that 7.3%, 8.3%, 1.8% and 5.5% of enterotoxigenic strains were mixed in the form of SE(A+C), SE(A+C+D), SE(G+H) and SE(A+G+H) respectively.

In the present study, we tried to detect the presence of *Staph. aureus* and their ability for enterotoxin production in milk and some dairy products and their food handlers in Zagazig City, Sharkia Governorate Egypt.

Staph species was isolated from 59.5% of raw milk samples, 40% of ice-cream samples, 70% of kariesh cheese samples and 80% of nasal swabs with statistical significant difference between colonized and non-colonized cases (P = 0.03). This is concomitant with **Rall *et al.*, (2008)**, who found that 70.4% of raw milk samples were contaminated with Staph organism, with **Awida (2009)** who found that 30% of ice-cream samples were contaminated with Staph organism, with **Hassan (2008),** who found that 72% of Kariesh cheese samples were contaminated with Staph organism, and lastly **Collery *et al.*, (2008)**, who found that 100% of nasal samples were contaminated with Staph. Organism.

From raw milk samples; *Staph. aureus* was isolated from 72.7% of animal samples, 87.5% of dairy farms samples, 83.3% of dairy shops samples and 88.9% of street distributors' samples. All of them

were exceeding Egyptian Standards, with significant statistical difference in the count of these colonies between different types of samples ($P < 0.001$). So, the highest percentage of samples with counts exceeding the Egyptian Standards was also observed in milk taken from dairy farms. The frequency distribution of the positive examined milk samples based on their *Staph. aureus* count/ml was nearly similar to that postulated by **Awida (2009)**, where the highest frequency distribution lies below 10 in the examined raw milk samples taken from dairy farms (59.2%), dairy shops (55.5%) and street distributers (93.3%).

The percentage of Staph species in ice-cream samples from street vendors (50%) was more than that of small scale producers (30%) and the maximum count was higher in street vendors (4×10^3) than in small scale producers (2×10^2) with significant statistical analytical result ($P < 0.03$), all of them exceeds the Egyptian Standards. Totally 87.5 % was *Staph. aureus*. This result was higher than the results obtained by **Ali (2000)**, 44% and **Hammad (2004)**, 52%.

For the kariesh cheese samples: colonization of street distributers (80%) was higher than that of grocery shops (60%) and the maximum count was higher in street distributers 5×10^6 than in grocery shops 2×10^5, all of them were exceeding the Egyptian Standards which is against the **Egyptian Standards (2005)**, that pointed out that the kariesh cheese must be free from *Staph. aureus*. 92.8% of cases were *Staph. aureus*. This percentage was nearly similar to that postulated by **Tawfeek *et al.*, (1988)**, **Hassan (2008)** and **Awida (2009)**, who could isolate *Staph. aureus* from 66%, 72% and 100% of the examined kariesh cheese samples respectively.

Nasal carriage is considered as a primary source of contamination of manually handled food as well as a source of *Staph. aureus* transmission between human. 80% of cases were colonized with Staph species, 50% were CPS and 50% were CNS, with significant statistical differences between colonized and non colonized cases. This is concomitant with **Collery *et al.*, (2008)**, who found that approximately, 20% of healthy human were estimated to be persistently colonized by *Staph. aureus*, while as many as 60% could be colonized intermittently.

As a whole, there were significant statistical differences between different types of samples concerning colonization, colony count, percent of CPS and CNS. It was obvious that the highest percentage of suspected Staphylococci isolates was observed in nasal swabs (80%) followed by kariesh cheese (70%), raw milk samples (59.5%) and in ice-cream samples (40%). However, after performing coagulase and confirmatory tests for *Staph. aureus*, it was found that the highest percentage of CPS was observed in kariesh cheese (92.8%), followed by ice-cream samples (87.5%) and raw milk (84%) and the lowest percentage was observed in nasal swabs, (50%) of samples. Similar percentage of CPS among suspected *Staph. aureus* isolates in raw milk samples was postulated by **Awida (2009)**; 96.3%, in addition to the higher percentage of CPS in both ice-cream and kariesh cheese samples which was 100% out of *Staph. aureus* isolates.

It's interesting that; 49% of CPS isolated from milk samples were toxigenic. The highest percentage of toxigenic CPS isolated from milk samples was observed in milk taken directly from animal (68.75%) and the lowest percentage was in milk observed from dairy shops (32%), with significant statistical difference (P value=0.04). As regard ice-cream samples, 42.86% of CPS was toxigenic and there was no significant difference between the percentage of enterotoxigenic *Staph. aureus* among CPS isolates in ice-cream samples from the street vendors (40.91%) and small scale producers (46.15%) (P value=0.693). As regard kariesh cheese samples, enterotoxigenic *Staph. aureus* was detected in 53.85% of CPS isolated from kariesh cheese samples. The percentage of enterotoxigenic *Staph. aureus* in kariesh cheese from street distributers (65.79%) was higher than that in kariesh cheese from grocery shops (37.04%) and the difference was statistically significant ($P = 0.001$). Also, 50% of CPS isolates from nasal swabs of food handlers were enterotoxigenic. Nearly similar result was observed by **Bania *et al.*, (2006)** and ***Chapaval et al.*, (2006)**, who found out that 54% and 44.54% respectively of the detected CPS isolates in milk and milk products samples produced enterotoxins. Although the selected 220 isolates from milk, milk products and nasal swabs were strongly producing coagulase, only 109 (49.5%) were enterotoxigenic which confirm what was stated by **Ryser (2001)**, that attempts to associate enterotoxin production by *Staph. aureus* with specific biochemical properties generally failed. Consequently, confirmation of the toxin by SDS-PAGE or other means provide the only proof that the particular strain is enterotoxigenic. The PCR technique was applied to all 109 toxigenic strains of *Staph. aureus* isolated from different samples. In milk and dairy products, the major classical enterotoxin genotype was SEA which was detected in 29 isolates (29.3%). SEC was detected in 16 isolates (16.1%) and SED was detected in 10 isolates (10.1%). For the newly described genes, SEG was detected in 10 isolates (10.1%) followed by SEH which was detected in 7 isolates (7.1%). The mixed forms were found in eight isolates (8.1%) for SE (A+C) genes another eight isolates (8.1%) for SE (A+C+D) genes, two isolates (2.02%) for SE (G+H) genes and five isolates (5.04%) for SE (A+G+H) genes. The higher percentage of SEA gene 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 animal origin than from of human origin. These strains of human origin contaminate milk and dairy products during

different stages of production and processing or even at consumer outlet. On the other side, the presence of SEC and SED can be attributed to the increased incidence of staphylococcal 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". Out of 10 toxigenic isolates of nasal swabs, the distribution of the classical enterotoxin genes was SEA in four isolates (40%), SEC in one isolate (10%) and SED in one isolate (10%). However, SEB could not be detected. For the newly described genes one isolate (10%) showed SEG and another one showed SEH gene. For the mixed forms, only one isolate (10%) showed SE (A+C) and another one showed SE (A+G+H). this is concomitant with ***Methotra et al.*, (2000)**, that could detect the toxin genes of *Staph. aureus* strains isolated from human nasal carriers.

Table 3. Percentage of enterotoxigenic strains of *Staph. aureus* among CPS isolated from different types of samples examined by SDS-PAGE

Examined Samples	No. of tested CPS isolates	Toxigenic No.	Toxigenic %
Raw milk samples			
(direct from animal)	16	11	68.75
Dairy Farm	35	20	57.14
Dairy shops	25	8	32
Street distributers	24	10	41.67
Total	100	49	49%
P - value			<0.04
Ice- cream samples			
Street vendors	22	9	40.91
small scale producers	13	6	46.15
Total	35	15	42.8%
P - value			0.693
Kariesh cheese samples			
Street distributers	38	25	65.79
Grocery shops	27	10	37.04
Total	65	35	53.8%
P - value			< 0.001
Nasal swabs samples			
Nasal swabs	20	10	50%
Total	220	109	49.5%

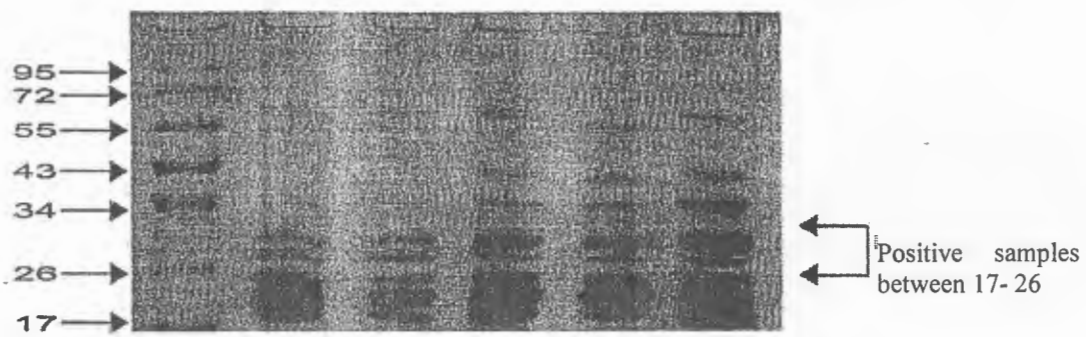

Photo (1): Gel stained with coomassie blue for protein visualization of *Staph. aureus* enterotoxins.

Lane 1: Marker (Page Ruler prestained protein ladder)
Lane 2: SEs detected in a milk sample (directly from animal)
Lane 3: SEs detected in an Ice-cream sample (street vendor)
Lane 4: SEs detected in a Kariesh cheese sample (street distributer)
Lane 5,6: SEs detected in nasal swabs samples (human food handlers)

Table 4. Types and prevalence of toxigenic genes in *Staph. aureus* isolated from different samples

Examined samples		Toxigenic isolates		Classical superantigen genes		New superantigen genes	
		SEA	SEB	SEC	SED	SEG	SEH
		No. (%)	No.%	No. %	No. %	No. %	No. %
Raw milk samples							
From animals	11	2 (18.2%)	- 0	2 (18.2%)	1 (9.1%)	2 (18.2%)	1 (9.1%)
Dairy farms	20	4 (20%)	- 0	3 (15%)	2 (10%)	3 (15%)	1 (5%)
Dairy shops	8	3 (37.5%)	- 0	2 (25%)	1 (12.5%)	1 (12.5%)	0
Street distributors	10	4 (40%)	- 0	1 (10%)	1 (10%)	0	1 (10%)
*Total	49	13 (26.5%)	- 0	8 (16.3%)	5 (10.2%)	6 (12.2%)	3 (6.1%)
P - value		<0.04		<0.03	0.703	0.425	0.375
Ice- cream samples							
Street vendors	9	2 (22.3%)	- 0	1 (11.1%)	1 (11.1%)	2 (22.2%)	1 (11.1%)
Small scale producers	6	2 (33.2%)	- 0	1 (16.7%)	1 (16.7%)	- 0	- 0
Total	15	4 (26.7%)	0	2 (13.3%)	2 (13.3%)	2 (13.3%)	1 (6.7%)
P - value		0.425		0.752	0.532	<0.04	0.295
Kariesh cheese samples							
street distributers	25	8 (32%)	0	4 (16%)	2 (8%)	1 (4%)	3 (12%)
Grocery shops	10	4 (40%)	0	2 (20%)	1 (10%)	1 (10%)	0
Total	35	12 (34.3%)	0	6 (17.1%)	3 (8.6%)	2 (5.7%)	3 (8.6%)
P - value		0.391		0.425	0.374	0.643	0.591
Total milk samples							
	99	29	0	16	10	10	7
Nasal swabs							
Nasal swabs	10	4	0	1	1	1	1
Total	109	33	0	17	11	11	8

Table 5. Percentage of mixed forms of superantigen genes

Examined samples	Toxigenic isolates	SE (A+C)		SE (A+C+D)		SE (G+H)		SE (A+G+H)	
		No.	%	No.	%	No.	%	No.	%
Raw milk samples									
from animals	11	1	9.1	-	0	1	9.1	1	9.1
dairy farms	20	2	10	1	5	-	0	2	10
dairy shops	8	1	12.5	-	0	-	0	-	0
street distributer	10	1	10	1	10	-	0	1	10
*Total	49	5	10.2	2	4.08	1	2.04	4	8.2
P - value		0.529		0.738		0.695		0.723	
Ice- cream samples									
street vendors	9	-	0	1	11.1	-	0	-	0
small scale producers	6	1	16.7	1	16.7	-		-	0
*Total	15	1	6.7	2	13.3	-	0	-	0

Table 5 Cont.

Kariesh cheese samples									
Street distributers	25	2	8	3	12	1	4	-	0
grocery shops	10	-	0	1	10	-	0	1	10
*Total	35	2	5.7	4	11.4	1	2.9	1	2.9
P - value		0.372		0.701		0.639		0.591	
Total milk samples									
	99	8	8.1	8	8.1	2	2.02	5	5.04
Nasal swabs									
Nasal swabs	10	-	0	1	10	-	0	1	10
Total	109	8	7.3	9	8.3	2	1.8	6	5.5

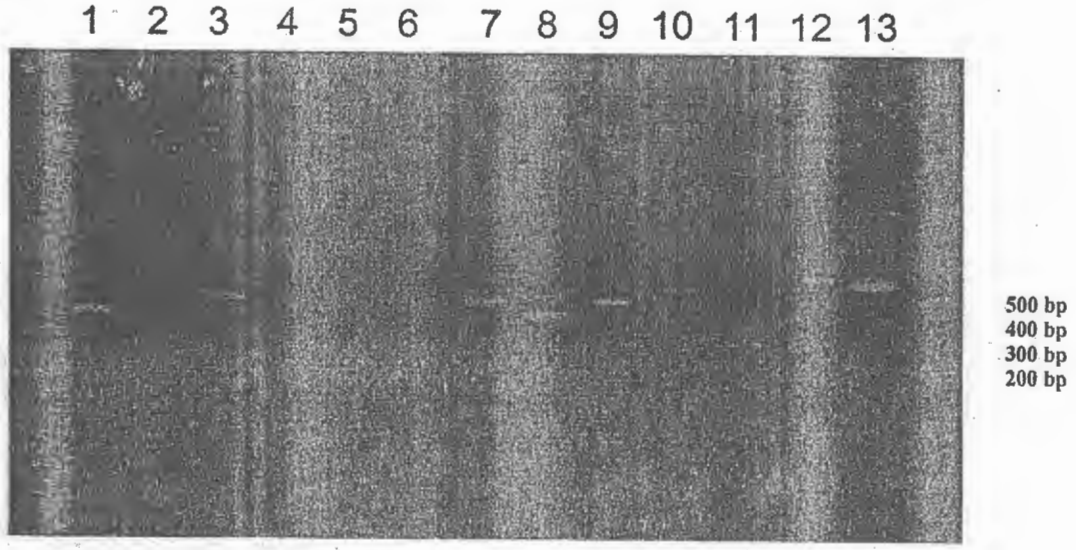

Photo (2): **Agarose gel electrophoresis of amplified superantigen genes.**
Lanes land S: Positive for SEG (328 bp).
Lanes 2,4,5,6 and 10: Negative samples.
Lanes 3, 7 and 9: Positive for SEH (359 bp)
Lane 11: Positive sample for SEC (284 bp).
Lane 12: Positive sample for SEA, (521 bp).
Lane 13: Marker (100 - 1000 bp).

References

Ali, M. M. (2000): "Studies on enterotoxigenic Staphylococci in milk and dairy products". Ph. D. thesis, Fac. Vet. Med., Cairo Univ., Egypt.

A.P.H.A. (1992): "Standard Methods for the Examination of Dairy products". 16th Ed., American Public Health Association. New York.

Astrid, L.; Semir, L.; and Knut, G. (2004): "Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates". Section of Feed and Food Microbiology, National Vetrinary Institute. J. of Clin. Microbiol.; 42: 3869-3872.

Awida, R.M. (2009): "Some studies on *Staph. aureus* in milk and some milk products sold in Assiut City with special reference to antibiotics resistant *Staphylococcus aureus*". Ph. D. Thesis, Fac. Vet. Med., Assiut Univ., Egypt.

Bania, J.; Dabrowska, A.; Bystron, J.; Korzekwa, K.; Chrzanowska, J.; and Molenda, J. (2006): "The profiles of enterotoxin genes in *Staph. aureus* from nasal carriers". Appl. Microbiol., 42: 315-320.

Bendahou, A.; Abid, A.; Bouteldoun, N.; Catelejine, D. and Mariam, L. (2009): "Enterotoxigenic - coagulase positive *Staphylococcus* in milk and milk products". J. Infect. Developing Countries; 3: 169-176.

Chapaval, L.; Moon, D.H.; Gomes, J.E.; Duarte, F.R. and Tsai, S.M. (2006): "Use of PCR to detect classical enterotoxins genes and toxic shock syndrome toxin-1 gene in *Staph. aureus* isolated from crude milk and determination of toxic

productivities of *Staph. aureus* isolates harboring these genes". Arq. Inst. Boil.; 73: 165-169.

Chiang, Y.C; Liao, W.W.; Fan, C.M.; Pai, W. K; Chiou, C.S. and Tsen, H.Y. (2008): "PCR detection of Staphylococcus enterotoxins N, O, P,Q,R,U and survey of SE types in *Staph. aureus* isolates from food poisoning cases in Taiwan". Int. J Food Microbiol.; Jan. 15;121(1) : 66-73.

Collery, M.M.; Smyth, D.S.; Twohig, J.M.; Shore, A.C.; Coleman, D.C. and Smyth, C.J. (2008): "Molecular typing of nasal carriage isolates of S. aureus from an Irish university student population based on toxin gene PCR, AGR locus types and multiple locus variable number tandem repeat analysis". J. Med. Microbiol., 57: 348-358.

Egyptian Standards (2005): "Milk and Dairy products: Soft cheeses". Egyptian Organization for Standardization and Quality Control; E.S.: 1008.

FAO (Food and Agriculture Organization) (1992): "Manual of Food Quality Control". 4 Rev. 1. Microbiological Analysis. Washington, D.C.; p.131-151.

Finegold, S.M. and Martin, W.J. (1994): "In Bailley and Scott Diagnostic Microbiology". 9th Ed., C.V. Mosby Co., St. Louis, Toronto, London, p.321.

Hammad, A.M. (2004): "Microbiological studies on raw milk and some dairy products". M. V. Sc. Thesis, Fac. Vet. Med., Sadat City, Minufiya Univ., Egypt.

Hassan, W.S. (2008): "Microbiological evaluation of some white soft cheese locally manufactured and sold in Assiut city". M. V. Sc. Thesis, Fac. Vet. Med., Assiut Univ., Egypt.

Hata, E.; Katsuda, K.; Kobayashi, H.; Nishimori, K.; Uchida, L; Higashide, M.; Ishikawa, E.; Sasaki, T. and Eguchi, M. (2008): "Bacteriological characteristics of Staphylococcus aureus isolated from humans and bulk milk". J. Dairy Sci. Feb; 91(2): 564-9.

Jarraud S.; Mougel, C; Thiolouse, J.; Lina, G.; Meugnier, H.; Forey, F.; Nesme, X.; Etienne, J. and Vandensech, F. (2002): "Relationships between S. aureus genetic background, virulence factors, alleles and human disease". Infect. Immun.; 70: 631-641.

Laemmli, U.K. (1970): "Cleavage of structural proteins during the assembly of the head of bacteriophage T4". Nature, 227: 680-685.

Leloir, Y.; Baron, F. and Gautier, M. (2003): "*Staph. aureus* and food poisoning". Genet. Mol. Res. 2: 63-76.

Macfadin, J. (1980): "Biochemical tests for identification of medical bacteria". 2nd Ed. Williams and Wilkins Co. USA, p. 365-370.

Mempel, M.; Lina, G.; Hojika, M. Schnopp, C.Seidl, H.P.; Schafer, T.; Ring, J.; and Vandenesch, F. (2008): "High prevalence of superantigens associated with the EGC locus in S. aureus isolates from patients with atopic eczema". Int. J. Clin. Microbiol. Infect. Dis., 22: 306-309.

Methotra, M.; Gehua, W. and Wendy, M. J. (2000): "Multiplex PCR for detection of Genes for *Staph. aureus* enterotoxins, exofoliative toxins, toxic shock syndrome toxin 1 and Methicillin Resistance". J. Clin. Microbiol.; 38: 1032-1035.

Omoe, K; Ishikawa, M; Shimoda, Y.; Hu, D.L.; Ueda, S. and Shinagawa K (2002): "Detection of SEG, SEH and SEI genes in *Staph. aureus* isolates and determination of enterotoxin productivities of *Staph. aureus* isolates harboring SEG, SEH or SEI genes". J. Clin. Microbiol.; 40: 857-862.

Rall, V.L.M.; Vieira, F.P.; Rail, R.; Vieitis, A.; Fernandes, J.R.; Candeias, J.M.C; Cardoso, K.F.G. and Araujo, j.R. (2008): "PCR detection of staphylococcal enterotoxin genes in *Staph. aureus* strains isolated from raw and pasteurized milk". Vet. Microbiol.; 132: 408-413.

Ryser, E.T. (2001): "Public health concerns. In Applied Dairy Microbiology". Marth, E.H. and Steele, J.L. (eds.). Marcel Dekker, Inc., New York, pp.: 478-486.

Srinivasan, V.;Sawant, A. A.; Gillespie, B.E.; Headrick, S. J.; Ceasaris, L. and Oliver, S.P. (2006): "Prevalence of enterotoxin and toxic shock syndrome toxin genes in *Staph. aureus* isolated from milk of cows with mastitis". Foodborne Pathog Dis. Fall; 3 (3): 274-83.

Taverna, F.; Negri, A.; Piccinini R.;Zecconi, A.; Nonnis, S.; Ronchi, S.; Tedeschi, G. (2007): "Characterization of cell wall associated proteins of a *Staph. aureus* isolated from bovine mastitis". Vet. Microbiol.; 119: 240-247.

Tawfeek, N.H.; Sharaf, O.M. and Hewedy, M.M. (1988): "Incidence of pathogens and staphylococcal enterotoxins in kariesh cheese". Egyptian J. Dairy Sci., 16: 295-300.

Thatcher, F.S. and Clark, D. S. (1975): "Microorganisms in foods". International Committee on Microbiological Specification for Foods. Univ. Toronto Press, Toronto, Canada,.

Wanger, A. R.; Morris, S.L.; Ericson, C; Singh, K. V. and Larcocco, M.T. (1992): "Latex agglutination. Negative methicillin resistant *Staph. aureus* recovered from neonates : Epidemiological features and comparison of typing methods". J. Clin. Microbiol.; 30:2583-2588.

Zschoch, M.; Kloppert, M.B.; Wolter, W.; Hamann, H.P. and Lammler, C. (2005): "Pattern of enterotoxin genes SEG SE, SEI and SEJ positive *Staph. aureus* from bovine mastitis". Vet. Microbiol.; 108:243-249.

الملخص العربى

الكشف عن السموم المعوية لميكروب ستافيلوكوكس أوريوس في الألبان ومنتجاتها والقائمين بتداولها في محافظة الشرقية

السيد محمد عبد الواحد

قسم علوم الأغذية – كلية الزراعة – جامعة الزقازيق

يحمل ميكروب ستافيلوكوكس أوريوس العديد من الجينات التي لها القدرة على إحداث تسمم غذائي وتؤدى الي نسبة كبيرة من الحالات الوبائية. هذا وقد كانت السموم المعوية الخمس المعروفة هى SEA إلى SEE، ولكن هناك أنواع جديدة تم التعرف عليها مثل SEG و SEH وهذه كلها يفرزها الميكروب في الألبان ومنتجاتها ومصدرها هو الإنسان أو الحيوان.

الهدف من البحث هو تقييم نسبة تواجد ميكروب ستافيلوكوكس أوريوس وسمومه في الألبان وبعض منتجاتها والتوصيف الجيني للسموم المعروفة وأيضا السموم التي تم التعرف عليها حديثاً.

تم جمع 450 عينة (200 من اللبن الخام و **100** من الآيس كريم و 100 من الجبن القريش) بالإضافة إلى 50 مسحة أنفية عشوائيا من المتداولين لهذه المنتجات بمدينة الزقازيق وبعض القرى التابعة لها – محافظة الشرقية – جمهورية مصر العربية. وقد تم فحصها بكتيريولوجيا باستخدام بيئة Baird-Parker agar لمعرفة نسبة تواجد ميكروب ستافيلوكوكس أوريوس، وتم التأكيد باستخدام اختبار إنزيم التجلط واختبار الجلتنة للكشف عن عامل التجمع وبروتين A والحافظة متعددة السكريات، وتم الفصل الكهربائي لبروتينات الميكروب باستخدام SDS-PAGE للتعرف على العزلات السامة. وقد استخدم تفاعل إنزيم البلمرة المتسلسل للتعرف على نوع وتوزيع الجينات الفائقة الخاصة بالسموم المعوية لميكروب ستافيلوكوكس أوريوس (A,B,C,D,G,H).

وقد تبين بالفحص البكتيريولوجي أن نسبة تواجد ميكروب ستافيلوكوكس أوريوس الموجب لإنزيم التجلط كانت ٨٤٪، 87.5٪، 92.8٪، 50٪ من عينات اللبن الخام والأيس الكريم والجبن القريش والمسحات الأنفية على التوالي، كما وجد أن 62.5٪ من العينات تجاوز عدد الميكروب بها النسبة المسموح بها بمصر. هذا وقد وجد أن 49.5٪ من السلالات الموجبة لإنزيم التجلط تمثل عزلات سامة، وقد وجد أن أعلى نسبة كانت في اللبن الخام المجمع من الحيوان مباشرة ومن الجبن القريش المجمع من البائعين الجائلين بنسبة 68.7٪، 65.7٪ على التوالي. بالنسبة للألبان ومنتجاتها، فقد كان تواجد الجين الخاص بالسم المعوي A هو الأكثر تواجداً حيث وجد في 33 عزلة، الجين الخاص بالسم المعوي C في ١٧عزلة، الجين الخاص بالسم المعوي D في **11** عزلة، بينما الجين الخاص بالسم المعوي B لم يتم التعرف عليه في أي من العينات. وكان من أنواع الجينات التي اكتشفت حديثا الجين الخاص بالسم المعوي G وقد وجد في 11 عزلة والجين الخاص بالسم المعوي H في 8 عزلات. وقد وجدت بعض الجينات معاً في نفس العزلة في 25 عينة بينما ٤ عزلات وجد أنها تحمل جينات غير موصفة.

الخلاصة أن نسبة كبيرة من الألبان وبعض منتجاتها وخاصة الجبن القريش المعروضة للبيع في محافظة الشرقية – جمهورية مصر العربية، ملوثة بميكروب ستافيلوكوكس أوريوس وسمومه وأكثر أنواع السموم تواجداً هو السم A وهو معروف أنه أقل تواجداً في المصدر الحيواني عن نظيره في المصدر البشرى. هذا وقد تبين أن البائعين الحاملين للميكروب في الغشاء المخاطي للأنف يعتبرون المصدر الرئيسي لتلوث الألبان ومنتجاتها.