Prevalence of Staphylococcus *aureus* and its enterotoxin in soft cheese.

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A B S T R A C T

A total of 180 random samples of marketed soft cheese samples classified package and retail (open) (Kareish, Domiati and Feta) (30 of each) were collected from different shops at El- Qualyubia Governorate to evaluate presence of *Staph. aureus*. Most of all examined cheese samples (Kareish, Domiati and Feta) were contaminated with *Staph. aureus* by different ratios. The obtained result revealed that (0,13.3, 0,10, 10 and 26.6%) respectively for packed and retail samples. It could be inferred that regarding the soft cheese contamination, retail Domiati cheese samples recorded the highest *Staph. aureus* contamination levels (10%).Retail Feta samples recorded the highest enterotoxigenic *Staph. aureus* contamination (37.5%). It is notable that only samples of retail kareish and retail Feta recorded enterotoxigenic *Staph. aureus* contamination, where *Staph. aureus* enterotoxin (SE) type D was only detected.

Key words: *Staph aureus*, enterotoxins, Kareish, Domiati, Feta cheese.

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1. INTRODUCTION

White soft cheese is one of the common delicious cheeses consumed in Egypt as it has avital source of nutrition for many people; either for adults or children due to its contributions of high quality animal protein. Therefore, many bacteria including spoilage and pathogenic bacteria, can grow and propagate in it. (Gruetzmacher and Bradly, 1999; Hayes et al., 2001). The microbiological quality of dairy products is influenced by the initial flora of raw milk, the processing conditions, post-heat treatment contamination and storage, as well as, equipment and environmental hygiene during manufacturing, packaging and handling are greatly affected the microbiological quality of cheese (Rajagopal et al., 2005; Robinson and Tamime, 2002). Cheeses are more susceptible to spoilage due to microbial contamination. The sources of contamination include both intrinsic factors (nutrients, water activity, pH, temperature, inhibiting factors produced by starter cultures and non-starter microorganisms, (competitive microflora, etc.) and extrinsic factors (microbial quality of raw milk, production phases, ripening and packaging conditions, the presence of chemical preservatives, etc…), also by cheese vat, cheese cloth and curd cutting knife, production room air and storage room (Brito et al., 2008; Hosny et al., 2011; Prencipe et al., 2010; Temelli et al., 2006). The presence of *Staph. aureus* in cheese constitutes a potential public health hazard since many strains of *Staph. aureus* produce entero-toxins that cause food poisoning if ingested. Neither the absence of *Staph. aureus* nor the presence of small numbers is complete assurance that a food is safe. Conditions inimical to the survival of *Staph. aureus* may result in diminishing operation or death of viable microbial cells, while sufficient toxins remain to elicit symptoms of staphylococcal food poisoning (Lancette and Tatini, 1992). *Staphylococcus aureus* entero-toxins are the cause of the gastrointestinal symptoms observed during intoxications. *Staphylococcus aureus* is considered the third most important cause of disease in the world amongst the reported food-borne illnesses (Tamarapu et al., 2001). The most common symptoms are nausea, vomiting and diarrhea. However, in severe cases they may be accompanied by acute prostration and abdominal cramps. Symptoms are usually occurring 2 to 6 h after ingestion of the contaminated food (Lancette and Tatini, 1992).

Therefore, the current study was carried out to evaluate the microbiological quality of soft cheese

sample sold under market conditions (packed and retailed) at Qualyubia Governorate area for isolation and identification of *Staph. aureus* and detection of *Staph. aureus* enterotoxins

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 180 random samples of marketed soft cheese samples (kareish, Domiati and Feta) classified retail and package (30 of each) were collected from different shops at El- Qualyubia Governorate. The collected samples were transferred directly to the laboratory in an ice box under complete aseptic conditions without undue delay and then subjected to the following bacteriological examination.

2.2. Bacteriological examination

2.2.1. Preparation of samples (British Standards Institution (BSI), 1984)

Ten grams of soft cheese sample were put in a sterile plastic bag and 90 ml of 2% sterile solution of sodium citrate were added at 45˚C ±1. The mixture was homogenized for 2 min. Thus, the cheese samples and sodium citrate are equal 10^{-1} dilution. Then, tenth fold serial dilution were prepared using sterile fourth strength ringer's solution.

2.2.2. Enumeration and identification of Staph aureus

2.3. Serological identification for Staph aureus and its enterotoxins (Oxoid, 1990):

Staphylase (using Oxoid Dry Spot staphytect plus kit) is a reliable latex slide agglutination test for detection a wide range of *Staph. aureus* strains. The dry spot kit (DR100M) is composed of DR101M dry spot staphytect plus reagent cards and Blue Latex Particles Coated with both procaine fibrinogen and rabbit IgG together with specific polyclonal antibodies raised against capsular poly saccharides of *Staph. Aureus.* About 50 µl of saline (0.9%) was added to small ring (at the bottom of each oval) in both of the test and control reaction areas ensuring that the liquid does not mixed with latex reagents. The sterile loop was used to pick up the equivalent of five average size suspected colonies from a culture media plate, emulsified carefully in the saline drop to give a smooth suspension. The suspension was mixed into the dry latex spots until completely suspended and spread to cover the reaction area using the loop. The card was picked –up and rocked for up to 20 seconds and observed for agglutination under normal lightening condition. When the test was completed, reaction cards were disposed of safely into disinfectant. If agglutination of the latex particles occurs within 20 seconds, this indicates the presence of *Staph. aureus*. If no agglutination occurs and a smooth blue suspension remains after 20 seconds in the test area, this indicates that the isolates were not *Staph. aureus*.

2.4. Detection of Staphylococcus aureus enterotoxins

Isolated *Staph. aureus* strains were examined for their ability to produce enterotoxins using staphylococcal enterotoxin–reverse passive latex agglutination Kit (SET-RPLA) and sac culture method (Donnelly et al., 1967): Dialysis tubes 50 cm long pieces and 3cm width were washed with distilled water, one end of each tube was knotted and the tube inflated to make a sack. The knotted end of the sac was inserted into 250 ml Erlen meyer flask to rest on the bottom of the flask, then 50 ml of double strength BHI broth were placed into the sac and the open end was knotted. The two knotted ends of the dialysis sac were tied together with a tuber band and the sac was placed in the flask in u-shape with knotted ends located to the neck. The flask and the contents were autoclaved at 121ºC for 15 minutes. Any excess liquid in the flask was removed. The growth of each strain was collected from BHI agar slant previously inoculated and incubated for 18-24 h at 37ºC. harvested with 2ml sterile saline. Eighteen ml of sterile phosphate buffer saline (0.2 M sodium phosphate, PH 7.4, in 0.9% sodium chloride), were inoculated with the harvested culture and then the mixture was transferred to Erlenmeyer flasks containing the sacs. The flasks were incubated in electric shaker bath (200 RPM) at 37 ºC for 48 hr. The culture fluid was removed from the flasks and centrifuged for 15 minutes at 23.800 xg to obtain a clear culture supernatant fluid. The clear culture supernatant fluid was tested serologically by RPLA technique using SET-RPLA (Oxoid) (A Kit for the detection of Staphylococcal Enterotoxins A, B, C and D) (manufactured by Denka Sekeu LTD., Japan for Oxoid LTD.) (Oda et al., 1979; Shingaki et al., 1981).

3. RESULTS

Mean values of *Staph. aureus* in retail Kareish, retail Domiati, packed Feta, retail Feta with $7.3x10^2 \pm 4.4x10$, $3.15x10^3 \pm 5.35x10^2$, $4.4x10^2 \pm 8.7x10$ and $1.6x10^3 \pm 2.05x10^2$, respectively as showen in (Table,1). While, it has been failed to detect *Staph. aureus* in samples of Packed kareish and Packed domiati. In addition, enterotoxiginic

strains of isolated *Staph. aureus* (Table, 2 and 3) detected in 25% and 37.5% (incidence in relation to positive *Staph.aureus* samples) of retail kareish and retail feta, respectively were enterotoxiginic, while failed to detect enterotoxiginic *Staph. aureus* in samples of retail domiati and packed feta. Application of SET-RPLA technique to identify type of enterotoxin of isolated enterotoxiginic *Staph. aureus* revealed that all isolated strains were producing D-enterotoxin

Table (1): Count of *Staph. aureus* in examined cheese samples (n=30) (cfu/g).

Sample	No. of positive samples	%	Min	Max	Mean ± SE*
Packed kareish	Zero	Zero	---	---	----
Retail kareish	4	13.3	$6.5x10^2$	$8x10^2$	$7.3x10^2 \pm 4.4x10$
Packed domiati	Zero	Zero	---	---	----
Retail domiati	3	10	$1.8x10^3$	$5x10^3$	$3.15x10^3 \pm 5.35x10^2$
Packed feta	3	10	$2x10^2$	$7x10^2$	$4.4x10^2 \pm 8.7x10$
Retail feta	8	26.6	$1.1x10^3$	$2x10^3$	$1.6x10^3 \pm 2.05x10^2$
Total	18/180	10	$2x10^2$	$5x10^3$	$1.65x10^3 \pm 3.26x10^2$

* SE means Standard error

Table (2): Incidence of enterotoxigenic *Staph. aureus* isolated from cheese samples.

Type of sample	NO. of coagulase positive *Staph. aureus*	Number of +ve enterotoxigenic strains	
		NO.	%
Retail kareish	4	1/4	25
Retail domiati	3	0/3	0
Packed feta	3	0/3	0
Retail feta	8	3/8	37.5
Total	18	4/18	22.2

Table (3): Types of *Staph. aureus* enterotoxins in positive *Staph. aureus* contaminated cheese samples.

Positive *Staph, aureus* samples	No. of positive samples	Type of *Staph. aureus* enterotoxin				
		A	B	C	D	E
Retail kareish	1				+	
Retail feta	3				+	
Total	4				+	

Table (4): Acceptability of examined soft cheese samples in relation to (Egyptian Organization for standardization and Quality Control (EOS), 2005).

EOS*, 2005	Cheeses		Packed cheese				Retail cheese			
			Prevalence		Acceptability		Prevalence		Acceptability	
			Number	%	Number	%	Number	%	Number	%
Staph. aureus	Free	Kareish	Zero	0	30	100	4	13.3	26	86.6
		Domiati	0	0	30	100	3	10	27	90
		Feta	3	10	27	90	8	26.6	22	73.3

* EOS means Egyptian organization for standardization and Quality control

4. DISCUSSION

Staphylococcus aureus is an important food-borne pathogen and a major cause of food poisoning outbreaks worldwide. The presence of *Staph. aureus* in ready to eat food which are eaten without cooking could be a bacterial risk for humans (Odumeru et al., 1997). In the present study, staphylococcus species which detected in the examined white soft cheese samples (packed and retail) (Kareish, Domiati and Feta) were investigated bacteriologically to detect the occurrence of enterotoxigenic *Staph. aureus* in soft cheese.

Identification of isolated staphylococci revealed presence of *Staph. aureus* in retail Kareish, retail Domiati, packed Feta, retail Feta with incidence of 13.3,10,10 and 26.6 % (Table 2). With mean values $7.3x10^2 \pm 4.4x10$, $3.15x10^3 \pm 5.35x10^2$, $4.4x10^2 \pm 8.7x10$ and $1.6x10^3 \pm 2.05x10^2$, respectively. While, it has been failed to detect *Staph. aureus* in samples of Packed kareish and Packed domiati. Packed Kareish, retail Kareish, packed domiati, retail domiati, packed feta and retail feta samples were accepted by incidence of 100, 86.6, 100, 90, 90 and 73.3% according to Egyptian Organization for standardization and Quality Control (EOS) (2005) (Table, 4). In addition, enterotoxiginic strains of isolated *Staph. aureus* (Table, 2) detected in 25% and 37.5% (incidence in relation to positive *Staph. aureus* samples) of retail kareish and retail feta, respectively were enterotoxiginic, while failed to detect enterotoxiginic *Staph. aureus* in samples of retail domiati and packed feta. According to EOS, in kareish, domiati and feta cheese must be free from *Staph. aureus* and its toxins.

It's clear that the presence of *Staph. aureus* dairy products even in low numbers must regarded a public health hazard, because it has been established that *Staph. aureus* may lose its viability in food but its enterotoxins still exist. The viability of *Staph. aureus* during manufacturing of dairy products depends on the addition of starter culture, and storage time (Erkmen, 1995). Application of SET-RPLA technique to identify type of enterotoxin of isolated enterotoxiginic *Staph. aureus* revealed that all isolated strains were producing D-enterotoxin (Table, 3). EL-Malt (2013) detected 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 synthesis entertoxins B, entertoxins C, and enterotoxins D.

In the same context Rahimi (2013) found the ability to synthesize classical staphylococcal enterotoxins (SEA-E) was determined in 7 of 20 (35%) isolates by using ELISA. SE type C was the most common enterotoxin found in the isolated *Staph. aureus* (42.9%), followed by SE type A (28.6%), SEA+SEC and SE type D (14.3%). Of the 20 isolates, 16 (80.0%) were positive for one or more enterotoxin genes and 8 different genotypes were observed. On the other hand, Eid and El-talawy (2014) reported that out of 10 isolates of *Staph. aureus,* only 2 strains (20%), (1 each from) Tallaga and Kareish cheese were enterotoxigenic belonging to enterotoxin A and 2 strains (20%) belonging to A, C enterotoxin while the corresponding cheese samples were enterotoxins free.

It could be inferred that regarding Differences between the results may be based on the differences in the cheese production techniques, storage conditions, type of cheese and whether the milk used was raw or pasteurized. It could be also related to the unclean conditions where the cheese is produced and the personnel involved in production. Finally, there is a great need for rising up, developing and spreading the hygienic knowledge, attention and control measures where cheese is made, and handled.

5. REFERENCES

British Standards Institution (BSI), 1984. Microbiolgical examination of dairy purposes. BS4285: Section 2.1.

Brito, J.R.F., Santos, E.M.P., Areuri , E.F., Lange, C.C., Brito, M.A.V.P., Souza, G.N., 2008. Retail survey of Brazilian milk and Mina's freseal cheese and a contaminated dairy plant to establish prevalence, relatedness and source of Listeria monocytogenes isolates. Appl. Environ. Microbiol. 74, 4954-4961.

Donnelly, C.B., Leslie, J.E., Black, L.A., Lawis, K.H., 1967. Serological identification of enterotoxigenic Staph. aureus from cheese. J. Appl. Microbiol. 15, 917-924.

Egyptian Organization for standardization and Quality Control (EOS), 2005. Soft cheese. Part 3: domiati cheese , Part 4: Kareish cheese and Part 12 feta cheese. Es: 1008.

Eid, A.M., El-talawy, M.F., 2014. Enterotoxogenic staphylococcus aureus isolated from soft cheese. Benha Vet. Med. J. 27, 444-448.

EL-Malt, L.M., 2013. Occurrence of enterotoxigenic staphylococcus aureus in some cheese varieties in aswan city upper egypt. Assiut Vet. Med. J. 59, 139.

Erkmen, O., 1995. Behaviour of staphylococcus aureus in Turkish feta cheese during

manufacture and ripening. J. Food protection 58, 1201-1205.

Gruetzmacher, T.J., Bradly, R.L.J., 1999. Identification and control of processing variables that affect the quality and safety of fluid milk. J. Food Prot. 62, 625-631.

Hayes, M.C., Ralyea, R.D., Murphy, S.C., Carey, N.R., Scarlett, J.M., Boor, K.J., 2001. Identification and characterization of elevated microbial counts in bulk tank raw milk. J Dairy Sci 84, 292-298.

Hosny, I.M., El kholy, W.I., Murad, H.A., El Dairouty, R.K., 2011. Antimicrobial activity of curcumin upon pathogenic microorganisms during manufacture and storage of a novel style cheese "Karishcum". J. American Sci. 7 611-618.

Lancette, G.A., Tatini, S.R., 1992. "Staphylococcus aureus", In: C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, DC., p. 533-550.

Oda, T., Ohkubo, T., Nagai, M., Nishimoto, Y., Ohmoruk, K., 1979. Detection of Staphylococcal enterotoxins in foods by RPLA test. Ann. Rep. Funkauka City, Japan. Hyg. Lab. 4, 33-37.

Odumeru, J.A., Mitchell, S.J., Alves, D.M., Lynch, J.A., Yee, A.J., Wang, S.L., Styliadis, S., Farber, J.M., 1997. Assessment of the microbiological quality of ready-to-use vegetables for health-care food services. J. Food Prot. 60, 954-960.

Oxoid, M., 1990. E.Y. Bridson, ed. Unipath Ltd. Basingstoke, Hampshire England. Hand book of microbiological media for the examination of food by Ronald. M. Atlas.

Prencipe, V., Migliorati, G., Matteucci, O., Calistri, P., Giannatable, E.D., 2010. Valutazione della qualita igenico sanitaria di alcuni tipi di fornaggi prelavati in fase di ventida al dettaglion. Veterinaria Italiana 46, 221-231.

Rahimi, E., 2013. Enterotoxigenicity of Staphylococcus aureus isolated from traditional and commercial dairy products marketed in Iran. Braz J Microbiol 44, 393-399.

Rajagopal, M., Werner, B.G., Hotchkiss, J.H., 2005. Low pressure CO2 storage of raw milk: microbiological effects. J Dairy Sci 88, 3130-3138.

Robinson, R.K., Tamime, A.Y., 2002. Maintaining a clean working environment. In: Robinson, R. K. (Ed.), Dairy Microbiology Handbook, the Microbiology of Milk and Milk Products 3rd ed., Wiley, New York, p. 561-591.

Shingaki, M.H., Igarashi, H., Fujikawa, H., Ushioda, T., Terayama, T., Sakai, S., 1981. Study on reversed passive latex agglutination for the detection of staphylococcal enterotoxins A-C. Annu. Rep. Tokyo, Metropol. Res. Lab. Public Health 32, 128-131.

Tamarapu, S., McKillip, J.L., Drake, M., 2001. Development of a multiplex polymerase chain reaction assay for detection and differentiation of Staphylococcus aureus in dairy products. J Food Prot 64, 664-668.

Temelli, S., Anar, S., Sen, C., Akyuva, P., 2006. Determination of microbiological contamination sources during Turkish white cheese production. Food Control 17, 856-861.