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DETECTION OF ENTEROTOXIGENIC *STAPHYLOCOCCUS AUREUS* IN SOME MEAT PRODUCTS

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ABSTRACT

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Eighty four meat product samples (minced meat, sausage and beef burger 28 of each) were collected and examined for detection of *Staphylococcus aureus.* The mean count was $4.3x10^2$, $5.1x10^3$ and $8.7x10^3$ cfu / gm, respectively. Multiplex Polymerase Chain Reaction (PCR) was applied for detection of genes responsible for enterotoxins production from identified coagulase positive *Staphylococcus aureus*. The predominant genes were A and B. They were detected in examined sausage and beef burger samples with different percentages.

Key words: *Detection, Meat products, Staphylococcus aureus, Staphylococcal enterotoxins, Multiplex PCR.*

INTRODUCTION

Meat and meat products are considered the most important sources of food for people in every where as they supply the consumers with the required nutritive elements such as high quality proteins, essential amino acids, B-complex vitamins and certain minerals especially iron and phosphorous and beside that the meat has high calories value.

In comparison to fresh meat, meat products retain more consumers' palatability and easily preparation characteristics. Food safety is a global health goal and the food borne diseases take a major crisis on health. Therefore, detection of microbial pathogens in food is the solution to the prevention and recognition of problems related to health and safety (Velusamy *et al.,* 2010). *Staphylococcus aureus* (*S. aureus*) is a major cause of food borne intoxication and its presence in food constitutes an important problem for food processors, food service workers and consumers. The main reservoirs of *S. aureus* are humans and animals. Healthy people carry the organism in their nose and throat (50 %), on their hands (5-30 %), and in wounds. *S. aureus* can also colonize food contact surfaces, and it can become a persistent organism in slaughter houses. *S. aureus* can contaminate foods through contact with contaminated hands, materials and surfaces, but also via the air coughing (Rao *et al.,* 1980). Considering this hazard, meat and meat products should not be subjected to unnecessary contamination and they should be free from such serious pathogen to ensure a maximum margin of consumer safety.

S. aureus, if posses Staphylococcal enterotoxins genes may produce several enterotoxins (SEA to SEJ) (Monday and Bohach, 1999). Staphylococcal food poisoning (SFP) is one of the most prevalent causes of gastroenteritis worldwide. Symptoms of SFP have a rapid onset characterized by abdominal cramps, nausea, and vomiting, sometimes followed by diarrhea. Patients usually suffer from symptoms within 2-4 hours after ingestion of thermostable Staphylococcal enterotoxins (SEs). The approximate dose of SEs ranged from 0.1 to 1.0 mg/kg of body weight (Jorgensen *et al.,* 2003 and Stewart *et al.,* 2005). Since SEs are more stable than *S. aureus* bacteria, it is possible to test food product and obtain negative *S. aureus* culture results and positive SE tests. Today, up to 15 SEs are known; the last one discovered was the recently identified SEU (Letertre *et al.,* 2003a). Multiplex PCR assay for detection of Staphylococcal enterotoxins genes (SEA, SEB, SEC, SED and SEE) was developed and proved to be specific, sensitive, and rapid method. (Omoe *et al., 2002* and Zschock *et al.,* 2005). There fore the current study was planed to through light on the incidence and count of *S. aureus* followed by PCR identification of enterotoxigenic strains.

MATERIALS and METHODS

Sampling:
Eighty four meat product samples (minced meats, sausages and burgers) 28 of each were collected randomly from supermarkets and butchery shops at Sharkia province, Egypt, at different levels of sanitations. The collected samples were identified, packed in sterile polyethylene bags and transferred to the laboratory as soon as possible.

Methodology:
The collected samples were examined for detection of *S. aureus* by ordinary culture method followed by biochemical identification and examined for coagulase enzyme production and then the detected strains were subjected to DNA extraction which was

used as template of PCR amplification for detection of *S. aureus* enterotoxins genes.

Preparation of samples, (ICMSF, 1978):
Twenty five grams of each examined samples were transferred to a sterile polyethylene bags, and 225 ml of 0.1% of sterile buffered peptone water was aseptically added to the content of the bag. Each sample was homogenized in a blender at 2500 rpm for 1-2 minute to provide homogenate of 1/10 dilution. One ml from the original dilution was transferred to another sterile tube containing 9ml sterile buffered peptone water and mixed well to make next dilution, from which further decimal serial dilutions were prepared. Then, dilutions were subjected to the following examinations.

Count and isolation of *Staphylococcus aureus*, (APHA, 1992):
From the previously prepared decimal dilutions of the examined samples 0.1 ml was transferred and evenly spread on dry surface of Baired-Parker agar medium plates (Oxoid, 1998). Inoculated and control plates were incubated at 37º C for 48 hours and Staphylococci count was calculated and recorded. Each suspected colony (black shiny with narrow white margin and surrounded by clear hallow-zone extended into the opaque medium) was picked up and cultured on slope agar for further biochemical identification according to (APHA, 1992).

Identification of isolated *Staphylococci organisms:*

- Morphological characters:
Staining (microscopical examination):
Films were made from the pure culture of isolated organisms stained by Gram's stain and examined microscopically. Gram +ve, grapes shape, cocci were considered as positive Staphylococci.

- Biochemical reactions:
1- Catalase test.
2- Oxidation-fermentation test.
3- Coagulase test.
4- Mannitol test.

PCR detection of Staphylococcal enterotoxins genes:

Extraction of the DNA from Bacterial culture:
Bacterial DNA was extracted using GeneJET Genomic DNA Purification kit (Fermentase, USA # k0722) following the manufacture's protocols.

PCR assays:
Oligonucleotide sequences of SE genes' were listed in table (1). PCR amplification was performed in a thermal cycler (Thermal cycler: PTC-100 ™ programmable thermal controller, Peltier-Effect cycling, MJ, RESEARCH, INC.) with an initial denaturation of 94° C for 5 minutes; 35 cycles of amplification (denaturation at 94° C for 2 minutes, annealing at 57° C for 2 minutes, and extension at 72° C for 1 minute), with a final extension at 72° C for 7 minutes. Electrophoration was done using 1.5% agarose gel in 0.5% Trisacetate- EDTA (TEA) buffer, stained with ethidium bromide. The amplified products then were visualized using ultra violet lamp wherein, the images were taken.

Multiplex PCR for detection of selected Staphylococcal genes:
The reaction conditions for the multiplex PCR assay were optimized to ensure that all of the target gene sequences were satisfactorily amplified. The primers were designed to target the coding regions of the genes; care was taken to avoid areas of homology within the structural genes for the enterotoxins. The primers used in each set had almost equal annealing temperatures, which reduced the possibility of occurrence of unwanted bands originating from nonspecific amplification.

Table 1: Nucleotide sequences, gene locations, and anticipated sizes of PCR products for the *S. aureus* gene-specific oligonucleotide primers used in this study (Mehrotra *et al.*, 2000).

Gene	primer	Location within gene	Sequence (5` – 3`)	Size of amplified product
sea	GSEAR-1	349–368	GGTTATCAATGTGCGGGTGG	102 bp
	GSEAR-2	431–450	CGGCACTTTTTTCTCTTCGG	
seb	GSEBR-1	666–685	GTATGGTGGTGTAACTGAGC	164 bp
	GSEBR-2	810–829	CCAAATAGTGACGAGTTAGG	
sec	GSECR-1	432–455	AGATGAAGTAGTTGATGTGTATGG	451 bp
	GSECR-2	863–882	CACACTTTTAGAATCAACCG	
sed	GSEDR-1	492–514	CCAATAATAGGAGAAAATAAAAG	278 bp
	GSEDR-2	750–769	ATTGGTATTTTTTTTCGTTC	
see	GSEER-1	237–257	AGGTTTTTTCACAGGTCATCC	209 bp
	GSEER-2	425–445	CTTTTTTTTCTTCGGTCAATC	

RESULTS

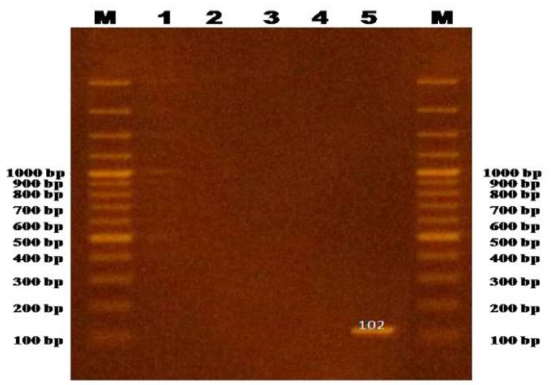
Table 2: Incidence and count of *S. aureus* in examined meat product samples and its percentage (n = 28).

Product	No of +ve samples	percentage	Min ′	Max ″	Mean**	± S.E*
Mince meat	6	21.4 %	<100	$10x10^3$	$4.3x10^{2\,b}$	$3.6x10^2$
Sausage	11	39.3 %	<100	$40x10^3$	$5.1x10^{3\,a}$	$2.1x10^3$
Burger	17	60.7 %	<100	$30x10^3$	$8.7x10^{3\,a}$	$1.8x10^3$

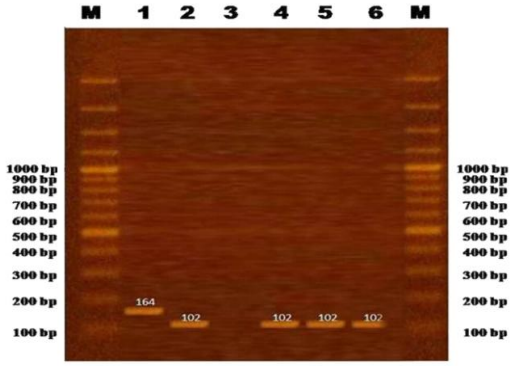
′ Min: minimum.
″ Max: maximum.
**Means carrying the different letter were significant; (P < 0.05).
* S.E: Standard error of mean.

Table 3: Incidence of enterotoxins of coagulase positive *S. aureus* detected in examined meat product samples and its percentage.

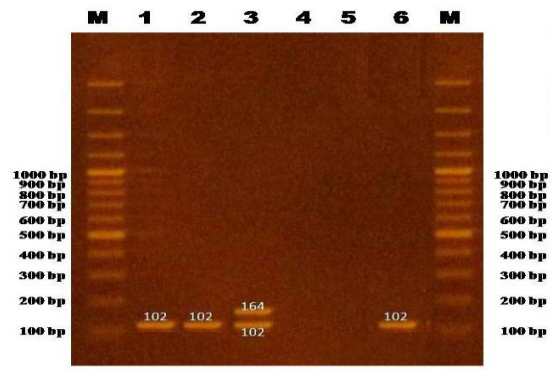
Product	No of +ve samples	% of +ve samples	Enterotoxin A		Enterotoxin B		Enterotoxins A&B	
			No	%	No	%	No	%
Minced meat	0	0 %	0	0 %	0	0 %	0	0 %
Sausage	9	32.1 %	8	88.9 %	1	11.1 %	0	0 %
Burger	8	28.6 %	6	75 %	1	12.5 %	1	12.5 %



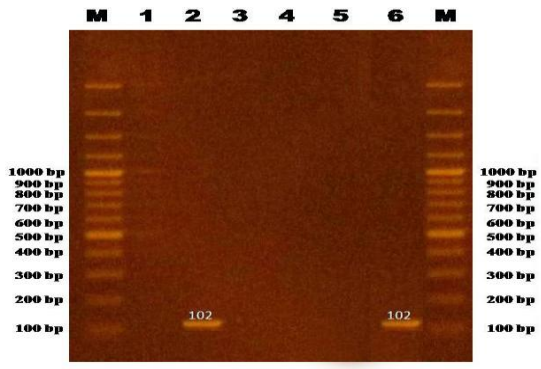
(1)



(2)



(3)



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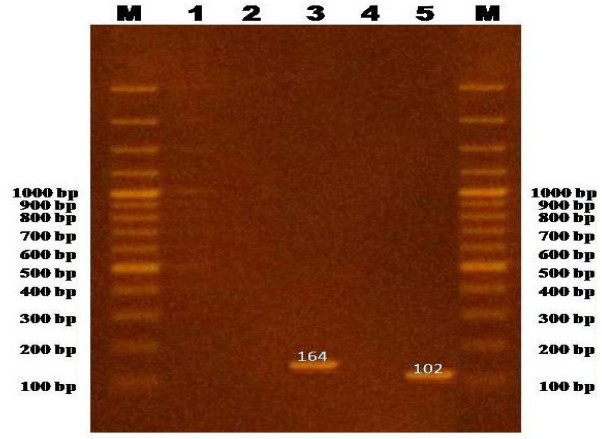

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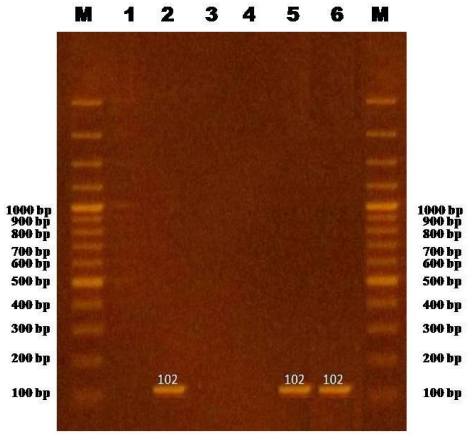

(6)

Figureure: Agarose gel electrophoretic pattern of:
- 1, 2 minced meat samples; 3, 4, 5 sausage samples.
- 3 minced meat samples; 1, 2,4,5,6 sausage samples.
- 1, 2 sausage samples; 3,4,5,6 beef burger samples.
- 6 sausage samples; 1, 2,3,4,5 beef burger samples.
- 1, 2, 4 minced meat samples; 3, 5 beef burger samples.
- 1, 2, 3, 4, 5, 6 beef burger samples.

DISCUSSION

***Staphylococcus aureus* count:**

Staphylococcal food-poisoning (SFP) is caused by Staphylococcal enterotoxins (SEs) produced during massive growth of *S. aureus* in food. SFP is a prevalent cause of food-borne disease worldwide, *(Jablonski and Bohach, 2001).*

The results showed in table (2) revealed that, the incidence of *S. aureus* in minced meat was 6 (21.4 %) similar result was recorded by Vorster *et al.* (1994) while lower incidence was detected by El-Said (2005). Higher incidences were recorded by Depourcq and Poucke, (1991); El-Gohary (1993); Ramasastry (1999); Salek (2000); Huffman (2002) and Tavakoli and Riazipour (2008). The mean count was $4.3x10^2 \pm 3.6x10^2$ cfu / gm and the count ranged from $<10^2$ to $10x10^3$ cfu / gm. Such results were lower than that recorded by Tharwat (2008) and Nossier (2010).

In sausage samples, the incidence was 11 (39.3 %). This incidence was similar to that obtained by EL-Sherbeeny *et al.* (1990) who detect *S. aureus* in 43.1% of examined sausage samples. On contrary lower incidences were reported by Abd El-Aziz *et al.* (1996); Soultos *et al.* (2003); El-Said (2005) and Gehad *et al.* (2006). Higher incidences were recorded by Rashad (1990), El-Gohary (1993) in the examined sausage samples.

The mean value was $5.1x10^3 \pm 2.1x10^3$ cfu / gm with a minimum of $<10^2$ and a maximum of $40x10^3$ cfu / gm. Lower results were obtained by *El-Mossalami, (2002)* who found that *S. aureus* count was <50 cfu/gm and *Gehad et al. (2006)* whom failed to detect *S. aureus* in the examined sausage samples.

Burger samples showed a high incidence of 17 (60.7 %), while lower incidences were reported by Ranucci *et al. (2004)* they isolated *S. aureus* from 21.1% of the examined hamburgers also El-Said (2005) recorded that *S. aureus* was isolated from frozen beef burger with an incidence of 2 (4%) and *Shahraz et al.* (2012) isolated 64 (24%) *S. aureus* strains from 256 samples of packaged hamburger. The mean value was $8.7x10^3 \pm 1.8x10^3$ organisms/ gm with a minimum of $<10^2$ and a maximum of $30x10^3$ organisms/ gm. Lower count was recorded by Essa and Makar, (2003).

Comparatively, the obtained results declared that burger samples gave a higher incidence of *S. aureus* followed by sausage then minced meat. This indicates that the unsanitary handling of the prepared meat products during processing, transportation and marketing. Also, was due to using a low quality meat and meat additives. The shape of burger may explain the higher incidence than that of sausage. Statistical analysis revealed that both sausage and burger were significantly higher than minced meat at p value < 0.05.

The United Kingdom Public Health Laboratory Service in 2000 classified meat products to four categories satisfactory, acceptable, unsatisfactory and unacceptable according to number of *S. aureus* as < 20, 20 to <100, 100 to $<10^4$ and $\geq 10^4$ CFU/g, respectively. We found that, all examined minced

meat samples were within the permissible limit and considered acceptable except only one sample that show unsatisfactory level of contamination ($10x10^3$ organisms/ gm).The examined samples of sausage and burger showed unsatisfactory level of contamination and were 11(39.3 %) and 17 (60.7%) for sausage and burger.

PCR assays used to identify the pathogen and its enterotoxin genes in food samples. The PCR assays could be made in hours rather than days, with a high sensitivity and method accuracy, allowing for the detection of very low concentrations of micro-organisms (Najera-Sanchez *et al.*, 2003).

Data in table (3) revealed that the incidence of identified enterotoxigenic *S. aureus* isolated from meat product samples by PCR technique was 9 (32.1 %) from sausage and 8 (28.6 %) from burger, while it couldn't be detected from minced meat. Nearly similar result was detected by Pinto *et al.* (2005). They found that forty out of 131 isolates (31%) tested were positive for enterotoxins production. Lower incidence of enterotoxins was detected by Kevin *et al.* (2009). They examined 155 samples of fermented pork product, and found that 39.35% of the samples were positive for *S .aureus*, but none were positive for the Staphylococcal enterotoxins. Higher incidence of enterotoxigenic *S. aureus* was detected by Oh *et al.* (2007) they found that 47% of the isolated *S. aureus* strains from the contaminated meat product were enterotoxigenic *S. aureus*. Also, El-Shater (2010) detect enterotoxigenic *S. aureus* with an incidence of (55.6 %) in sausage samples and Aydin *et al.* (2011) found that out of 147 *S. aureus* isolated from different foods including sausage, 92(62.6%) were enterotoxigenic *S. aureus*.

Enterotoxins are a group of serologically distinct proteins (A, B, C1–3, D, E and F) that are the causative agents of Staphylococcal food poisoning. Although their exact mode of action has yet to be fully elucidated, they are believed to stimulate an enteric-vagus nerve reflex triggering the vomiting centers of the brain (Sears and Kaper, 1996; Arbuthnott *et al.*, 1990). The enterotoxins can also act as super antigens, stimulating T lymphocytes to release cytokines and T-cell proliferation (Balaban and Rasooly, 2000; Krakauer, 1999).

The predominant enterotoxins isolated from examined sausage samples were enterotoxin A 8 (88.9 %) figures (1, 2, 3 and 4) detected at 102 bp then enterotoxins B 1 (11.1 %) figure (2) detected at 164 bp. Application of PCR assays for detection of enterotoxin production from coagulase positive *S. aureus* isolated from beef burger samples revealed that 6 (75 %) enterotoxin A detected at 102 bp figures (3, 4,5 and 6), followed by enterotoxin B figure (5) detected at 164 bp. With a percentage of 1 (12.5%) and enterotoxins A and B figure (3) detected in one sample at 102 and 164 bp. With a percentage of (12.5%). Such results substantiate what have been reported by *Hwang et al. (2007); Ruzickova et al. (2008)*; *El-Shater (2010) and Aydin et al. (2011)*.

Finally we conclude that:
Not all coagulase positive *S. aureus* are enterotoxigenic, since minced meat samples were negative, only nine sausage samples from eleven coagulase positive were enterotoxigenic (82 %) and only eight burger samples from seventeen coagulase positive were enterotoxigenic (47 %).

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الكشف عن وجود ميكروب المكور العنقودى الذهبى المفرز للسموم المعوية فى بعض منتجات اللحوم

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تُعد منتجات اللحوم مصدراً هاماً من مصادر البروتين الحيوانى نظرا لإحتوائها على العديد من العناصر الغذائية مثل الفيتامينات والأملاح المعدنية وكذلك الحموض الأمينية الأساسية اللازمة لبناء جسم الإنسان. ونظرا للزيادة المطردة فى زيادة تعداد السكان فقد تم التوسع فى صناعة منتجات اللحوم والإهتمام بنقل التكنولوجيا الحديثة فى تصنيع تلك المنتجات فى صور مختلفة منها شرائح اللحم واللحم المفرى والسجق والبرجر. تتعرض اللحوم ومنتجاتها للتلوث بالكثير من الميكروبات وخاصة بميكروب المكور العنقودى الذهبى ، يحدث ذلك خلال الإنتاج والتصنيع والمعالجة، حيث أن اللحوم ومنتجاتها وسط جيد للنمو والتكاثر وإفراز السموم أيضاً ، وبذلك تصبح مصدر للتسمم الغذائى للإنسان. يعتبر الميكروب العنقودي الذهبي من الميكروبات المهمة جدا وذلك لقدرته على التضاعف والإنتشار السريع وكذلك قدرته على إفراز مواد سامة حيث زادت عوامل الضراوة من قدرته علي الإنتقال خصوصا من خلال الأطعمة الملوثة. ولذا إستهدفت هذه الدراسة تحديد وتصنيف الميكروب العنقودي الذهبى بإستخدام طرق ظاهرية وأخرى جينية وذلك بإستخدام التكنولوجيا الحيوية (تفاعل انزيم البلمرة المتسلسل) لتحديد طرق الإنتقال خصوصا أثناء التسمم الغذائي · تم فى هذه الدراسة جمع 84 عينة من منتجات اللحوم (لحم مفرى، سجق و برجر بقرى 28 عينة من كل منها). تم فحص هذه العينات لمعرفة مدى وجود الميكروب المكور العنقودى الذهبى فيها؛ وجد أن متوسط الأعداد كالتالى 2.1 ± 10^{3} x 5.1 ، 10^{2} x3.6 $\pm10^{2}$ x4.3 10^{3} و 8.7 x 10^{3} $\pm$ 1.8 x 10^{3} / جرام من العينات المفحوصة على التوالى. أستخدم تفاعل البلمرة المتسلسل المتعدد (Multiplex PCR) لتحديد مدى وجود الجين المسئول عن إنتاج السموم المعوية من العترات المعزولة والتى أعطت نتيجة إيجابية فى إختبار التلزن. وقد تم الكشف عن الجينين أ ، ب والتى تم عزلهما من عينات السجق والبرجر البقرى بنسب مختلفة. نخلص من ذلك لنتيجة مهمة فى نهاية هذا البحث وهى أنه ليس كل العترات الموجبة لإختبار التلزن من ميكروب المكور العنقودى الذهبى قادرة على إفراز السموم المعوية وإحداث التسمم.