DETECTION OF *STAPHYLOCOCCUS* AUREUS CLASSIC ENTEROTOXIN GENES IN SOME MEAT PRODUCTS USING MULTIPLEX PCR.

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ABSTRACT

A total of 200 meat product samples of beef burger, sausage, minced meat and luncheon were randomly collected from different shops and markets in El-Gharbia and Kafr-El-Sheikh governorates. The samples from 3 brands A, B and C were examined for the presence S. aureus using classical bacteriological and molecular methods. The obtained results revealed that the 2, 1 and 4 samples of beef burger, sausage and minced meat respectively, were positive for S. aureus by PCR. Additionally multiplex PCR was used for detection of five S. aureus virulence classic enterotoxin genes (sea, seb, sec, sed and see). The obtained results revealed that the beef burger samples harbored (1) seb gene; meanwhile sausage sample harbored (1) sea gene and minced meat samples harbored (2) sea, (1) sec and (1) sed genes. This study offers the basis for further phenotypic and molecular characterization S. aureus isolated from these products to guarantee safe consumption of meat products.

Key words: *S. aureus*, meat products, virulence genes and PCR.

INTRODUCTION

Foods of animal origin including meat are required to maintain the health of a human body ***(Nestle, 1999).*** Meat and meat products are concentrated sources of high quality protein and their amino acid composition usually compensate for shortcomings in the food. They supply easily absorbed iron and assist the absorption of iron from other foods as well as zinc; they also are rich sources of B-complex vitamins including thiamin, riboflavin, niacin, biotin, vitamins (B6 and B12), pantothenic acid and folacin.

S. aureus can contaminate foods by extensive handling and cause illness in humans when ingested**,** so it is considered as a well known microorganism that implicated in food borne illness ***(Prange et al., 2005).*** Convenience food offers a suitable growth environment for *S. aureus*, which is able to grow and express virulence in a wide variety of foods such as mixed foods, meat and meat products.

Although *S. aureus* may produce a large variety of enterotoxins (A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, R and U), but 95% of poisoning outbreaks are caused by classical enterotoxins: A, B, C, D and E ***(Letertre et al., 2003).*** Staphylococcal enterotoxins are a leading cause of gastroenteritis and vomiting resulting from consumption of contaminated food. They are highly thermostable; normal cooking and pasteurization cannot totally inactivate them. So they cause food poisoning, and the onset of symptoms depends on amount of enterotoxin ingested. The importance to evaluate *S. aureus* pathogenic activity assessing the combination of virulence genes has been emphasized ***(Nagarajappa et al., 2012)***.

The identification of staphylococcal toxin genes in strains of *S. aureus* by the multiplex PCR offers a very specific, sensitive, relatively rapid and inexpensive alternative to traditional immunological assays which depend on adequate gene expression for reliability and sensitivity ***(Mehrotra et al., 2000)*.**

As information about the genetic variability of *S. aureus* isolated from different foods in El-Gharbia and Kafr-El-Sheikh governorates is little. Therefore**,** the goal of the present study was to investigate the presence of sea, seb, sec, sed and see enterotoxin genes in *S. aureus* isolated from some meat products using multiplex PCR technique.

MATERIALS AND METHODS

Collection of the samples:

A total of (200) meat product samples: beef burger (50), sausage (50), minced meat (40) and luncheon (60) were randomly collected from different shops and markets in El-Gharbia and Kafr-El-Sheikh governorates at different production dates. The collected samples from 3 brands (A, B and C) were transferred immediately using an ice box without undue delay to the laboratory where they were subjected to bacteriological and molecular examinations.

Preparation of samples:

Ten grams of each sample were aseptically put into sterile flask contained 90 ml of sterile peptone water (0.1%), homogenized using homogenizer for 2 minutes to provide a homogenate of 10^{-1} dilution. Then decimal serial dilutions were prepared up to 10^{-6} according to ***(APHA, 2001)*.**

Bacteriological examination:

Isolation of suspected *S. aureus* strains according to ***(APHA, 2001)*** and identification according to ***(Cruickshank et al., 1975)*.**

Molecular examination:

- **Polymerase Chain Reaction confirmation:**

  1- Bacterial DNA preparation was done according to ***(Wilson, 1987)*.**

  2- PCR amplification was done according to **(Pereira et al., 2010).**

  3- The PCR product visualization.

- **Multiplex PCR for the detection of enterotoxin genes (sea, seb, sec, sed and see) of *S. aureus* isolates:**

After DNA extraction by the pervious step, the amplification of enterotoxin genes of *S. aureus* was performed according to ***(Mehrotra et al., 2000)*** on a thermal cycler using the following conditions: an initial denaturation at 94°C for 5 min was followed by 35 cycles of amplification (denaturation at 94°C for 2 min, annealing at 57°C for 2 min, and extension at 72°C for 1 min), ending with a final extension at 72°C for 7 min. Amplified products were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator at 254 nm.

Table (1): Sequences of primers used for identification of enterotoxin genes (sea, seb, sec, sed and see) of *S. aureus:*

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
sea (F)	5′ TTGGAAACGGTTAAAACGAA′3	120	*Johnson et al., (1991)*
sea (R)	5′ GAACCTTCCCATCAAAAACA ′3		
seb (F)	5′ TCGCATCAAACTGACAAACG ′3	478	
seb (R)	5′ GCGGTACTCTATAAGTGCC ′3		
sec (F)	5′ GACATAAAAGCTAGGAATTT ′3	257	
sec (R)	5′ AAATCGGATTAACATTATCC ′3		
sed (F)	5′ CTAGTTTGGTAATATCTCCT ′3	317	
sed (R)	5′ TAATGCTATATCTTATAGGG ′3		
see (F)	5′ AGGTTTTTTCACAGGTCATCC ′3	209	*Mehrotra et al., (2000)*
see (R)	5′ CTTTTTTTTCTTCGGTCAATC ′3		

RESULTS

Table (2): Incidence of *Staphylococcus aureus* in different brands (A, B and C) of examined beef burger samples.

Brand	No. of examined samples	Positive samples according to colonial characters on Baired parker agar medium		Biochemically identified *S. aureus*		Molecular identified *S. aureus*	
		No.	%*	No.	%*	No.	%*
A	20	4	20	0	0	0	0
B	15	5	33.3	1	6.7	0	0
C	15	6	40	2	13.3	2	13.3

* The percentages were calculated according to the total number of examined samples.

Table (3): Incidence of *Staphylococcus aureus* in different brands (A, B and C) of examined sausage samples.

Brand	No. of examined samples	Positive samples according to colonial characters on Baired parker agar medium		Biochemically identified *S. aureus*		Molecular identified *S. aureus*	
		No.	%*	No.	%*	No.	%*
A	18	5	27.8	0	0	0	0
B	14	3	21.4	2	**14.3**	1	**7.1**
C	18	4	22.2	0	0	0	0

Table (4): Incidence of *Staphylococcus aureus* in different brands (A, B and C) of minced meat samples.

Brand	No. of examined samples	Positive samples according to colonial characters on Baired parker agar medium		Biochemically identified *S. aureus*		Molecular identified *S. aureus*	
		No.	%*	No.	%*	No.	%*
A	13	3	**23.1**	0	0	0	0
B	12	5	**41.7**	**3 (4 isolates)**	**25**	3	25
C	15	7	**46.7**	**1 (2 isolates)**	**6.7**	1	6.7

Table (5): Incidence of *Staphylococcus aureus* in different brands (A, B and C) of luncheon samples.

Brand	No. of examined samples	Positive samples according to colonial characters on Baired parker agar medium		Biochemically identified *S. aureus*		Molecular identified *S. aureus*	
		No.	%*	No.	%*	No.	%*
A	6	1	16.7	0	0	0	0
B	19	4	21.1	0	0	0	0
C	35	4	11.4	0	0	0	0

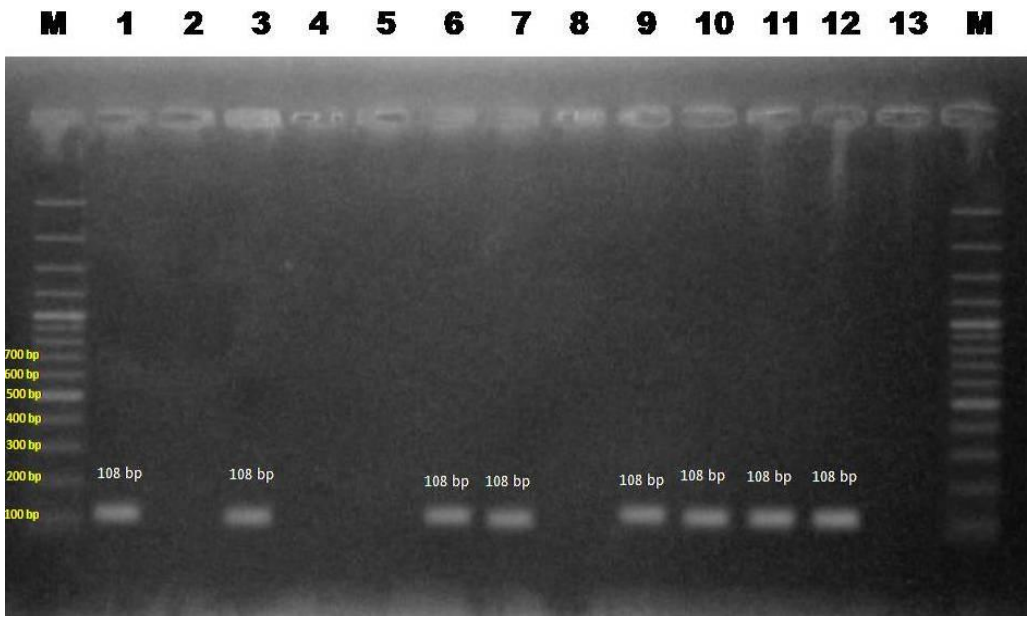

Fig. (1). Agarose gel Electrophoresis of PCR shows the results of identification of *S. aureus* (M= ladder 100 bp, lane 1 control positive, lane 2 control negative, lane 3 the isolated *S. aureus* from sausage sample, lane 4 negative sausage sample, lane 5 negative beef burger sample, lane 6 and 7 the isolated *S. aureus* from beef burger samples, lane 8 and 13 negative minced meat samples and lane 9,10,11,12 the isolated *S. aureus* from minced meat samples).

Table (6): Types of enterotoxin genes produced by the examined *S. aureus* isolates of positive samples.

Type of product	No. of positive samples	No. of *S. aureus* isolates	No. of examined isolates	Type and No. of SEs genes
Burger	2	3	2	(1) seb
Sausage	1	2	1	(1) sea
minced meat	4	6	4	(2) sea, (1) sec and (1) sed

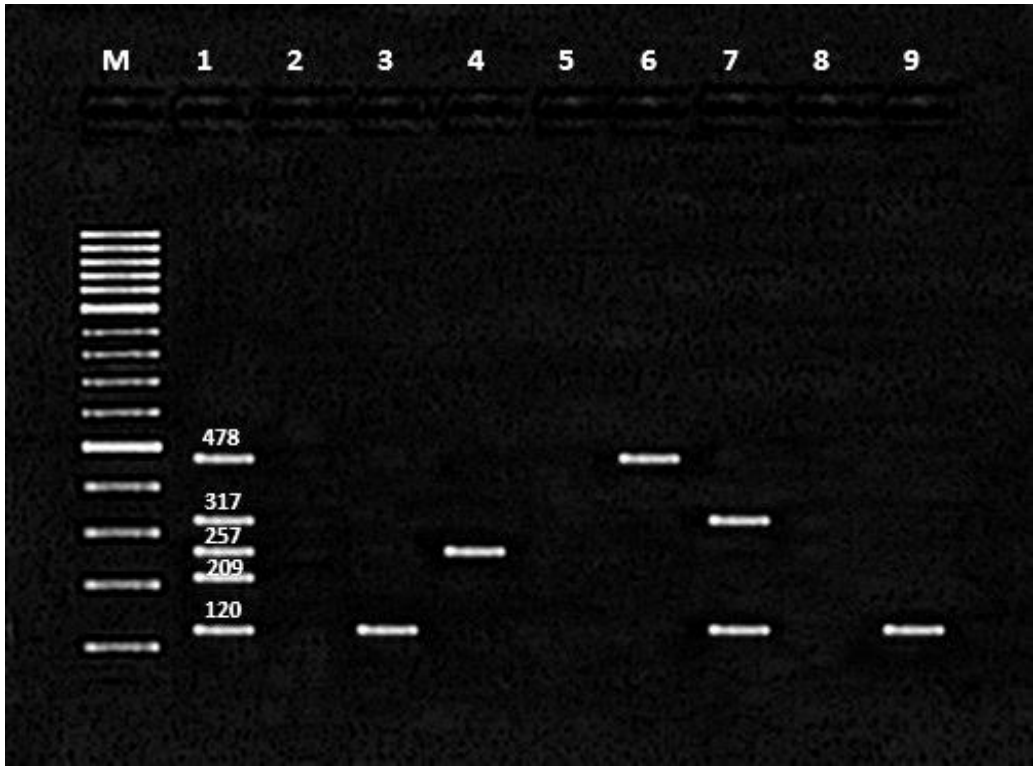

Fig. (2): Agarose gel electrophoresis of multiplex PCR showed the results for detection of *S. aureus* enterotoxin genes; (M= ladder 100 bp, lane 1 control positive of sea (120 bp), seb (478 bp), sec (257 bp), sed (317 bp) and see (209 bp) enterotoxin genes , lane 2 control negative, Lanes 3 and 9: Positive *S. aureus* strains for sea gene isolated from sausage and minced meat samples, Lane 4: Positive *S. aureus* strain for sec gene isolated from minced meat sample, Lane 6: Positive *S. aureus* strain for seb gene isolated from beef burger sample, Lane 7: Positive *S. aureus* strain for sea and sed genes isolated from minced meat sample and Lanes 5 & 8: Negative *S. aureus* strains for enterotoxins isolated from beef burger and minced meat samples).

DISCUSSION

Staphylococcus aureus is a leading cause of food poisoning resulting from the consumption of contaminated food with staphylococcal enterotoxins. Different foods can act as a good medium for *S. aureus* such as raw meat and meat products ***(Guven et al., 2010).***

From the results presented in table (2) it is evident that 20, 33.3 and 40% of brands (A, B and C), respectively of examined beef burger samples presumed to contain *S. aureus* (according to colonial character on Baird parker agar medium); while according to biochemical identification 0, 6.7 and 13.3% presumed to contain *S. aureus* and only 0, 0 and 13.3% of these examined samples were identified as *S. aureus* according to PCR.

On the other hand, the result presented in table (3) showed that 27.8, 21.4 and 22.2 % of brands (A, B and C), respectively of the examined sausage samples presumed to contain *S. aureus* (according to colonial character on Baird parker agar medium); while according to biochemical identification 0, 14.3 and 0 % presumed to contain *S. aureus* and only 0, 7.1 and 0 % of these examined samples were identified as *S. aureus* according to PCR.

Result presented in table (4) declared that 23.1, 41.7 and 46.7% of brands (A, B and C), respectively of the examined minced meat samples presumed to contain *S. aureus* (according to colonial character on Baird parker agar medium); while 0, 25 and 6.7 % were presumed to contain *S. aureus* according to biochemical identification and identified as *S. aureus* according to PCR.

While results in table (5) showed that 16.7, 21.1 and 11.4 % of brands (A, B and C), respectively of the examined luncheon samples presumed to contain *S. aureus* (according to colonial character on Baird parker agar medium). *S. aureus* was not detected in any of luncheon samples.

Results presented in table (6) declared that 2, 1 and 4 samples of beef burger, sausage and minced meat respectively, represented by 11 isolates were suspected positive for *S. aureus* and by PCR only 7 samples were positive as shown in (Fig. 1).

Also, the 7 samples as shown in table (6) and fig (2) were positive to *S. aureus* enterotoxin genes except for see gene by using multiplex PCR as follows: beef burger samples harbored (1) seb gene; meanwhile sausage samples harbored (1) sea gene and minced meat samples harbored (2)sea, (1)sec and (1) sed genes.

Among the increased demand of the meat products, it is of important to make these products of sanitary quality; they must be free from hazardous microorganisms or when present should be at a safe low level. The information given by the achieved results proved that most of the examined meat products brands (B and C) were contaminated with *S. aureus*. Also the incidences of hazardous microorganism like *S. aureus* is considered objectionable, as they render the product of inferior quality and unfit for consumption and this reflected that most of the workers were not bound to hygienic role of dressing clean clothes and regular washing of their hands during the different operations and this unhygienic personal conduct is a reflection of poor knowledge, practice and attitudes to meat handling ***(Mottin et al., 2011)***; while for luncheon samples the results were negative as luncheon subjected to heat treatment.

Staphylococcal enterotoxin A was mostly found in sausage and minced meat samples as shown in table (6) and this result is dangerous, because it is considered to be the most frequently occurring staphylococcal enterotoxin responsible for causing staphylococcal enterotoxicosis. *S. aureus* count higher than 10^5 CFU/g^{-1} is able to produce a quantity of enterotoxins that might cause food borne intoxication ***(Ercolini et al. 2004)*.**

REFERENCES

- **APHA (American Public HealthAssociation) (2001):** Compendium of methods for the microbiological examination of foods, 4th edition. American Public Health Association (APHA). Washington, DC USA.
- ***Cruickshank, R.; Duguid, J. P.; Marmion, B. P. and Swain, R. H. A. (1975):*** Medical microbiology. Churchilli Livingstone, Edinburgh 2, 119.
- ***Ercolini, D.; Blalotta, G.;Fusco, V. and Coppola, S. (2004):*** PCR-based detection of enterotoxigenic *Staphylococcus aureus* in the early stages of raw milk cheese making. J. Applied Microbiology. 96: 1090 – 1096.
- ***Guven, K.; Mutlu, B.M.; Gulbandilar, A. and Cakir, P. (2010):*** Occurrence and characterization of *Staphylococcus aureus* isolated from meat and dairy products consumed in Turkey. J. Food Safety, 30: 196-212.
- ***Johnson, W. M.; Tyler, S. D.; Ewan, E. P.; Ashton, F. E.; Pollard, D. R. and Rozee, K. R. (1991):*** Detection of genes for enterotoxins, exfoliative toxins, andtoxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. J. Clin. Microbiol., 29: 426–430.

- ***Letertre, C.; Perelle, S.; Dilasser, F. and Fach, P. (2003):*** Identification of a new putative enterotoxin SEU encoded by the egc cluster of *Staphylococcus aureus*. J. Appl. Microbiol., 95 (1): 38-43.
- ***Mehrotra, M.; Wang, G. and Johnson, W.M. (2000):*** Multiplex PCR for detection of genes for *S. aureus* enterotoxins, exfoliative toxins, toxic shock syndrome 1 and methicillin resistance. J. Clin. Microbiol., 38:1032-1035.
- ***Mottin, V.; Fisch, E. and Cardosa, M. (2011):*** Microbial contamination of luncheon meat sliced and packaged at supermarkets in Porto Alegre, Brazil , J. Acta Scientiae Veterinariae,39 (1): 940.
- ***Nagarajappa, S.; Thakur, M.S. and. Manonmani, H.K. (2012):*** Detection of enterotoxigenic staphylococci by Loop-mediated isothermal amplification method. J. Food. Safety, 32: 59-65.
- ***Nestle, M. (1999):*** "Animal v. plant foods in human diets and health: Is the historical record unequivocal ?". Proceedings of the Nutrition Society, 58 (2): 211-218.
- ***Pereira, P. M.; Atilano, M. L.; Yates, J.; Reed, P.; Veiga, H.; Pinho, M. G. and Filipe, S. R. (2010):*** Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in Staphylococcus aureus. Proceedings of the National Academy of Sciences**,** 107: 18991-18996.
- ***Prange, A.; Birzele, B.; Hormes J. and Modrow, H. (2005):*** Investigation of different human pathogenic and food contaminating bacteria and mould grown on Selenite/ Selenate and Tellurite / Tellurate by-x ray absorption spectroscopy.Food Control,16:713-728.
- ***Wilson, K. (1987):*** Preparation of genomic DNA from bacteria. Current protocols in molecular biology, pp. 2.4.1-2.4.5. Wiley, New York.

تحديد جينات السموم المعوية لميكروب المكور العنقودى الذهبي في بعض منتجات اللحوم بإستخدام تفاعل البلمرة المتعدد

تعتبر منتجات اللحوم من أهم مصادر الغذاء لما تحتويه من عناصر غذائية ضرورية لبناء الجسم ولكنها تعتبر من أكثر المصادر المسببة للتسمم الغذائي إذا تم معاملتها بطرق خاطئة من الناحية الصحية أثناء إنتاجها وتصنيعها وحتى وصولها إلى المستهلك .

ولهذا تم تجميع مائتي عينة عشوائية من بعض منتجات اللحوم (البرجر–السجق – اللحم المفروم –اللانشون) من مختلف المحلات والأسواق التجارية بمحافظتى الغربية وكفر الشيخ وذلك لفحصها ميكروبيولوجيا وجزيئيا للكشف عن مدى تواجد ميكروب المكور العنقودى الذهبى ثم الكشف عن الجينات المسببة للتسمم المعوي التى يفرزها.

كما أسفرت النتائج عن الاتى أن حوالى 7 عينات (2 من البرجرو 1 من السجق و 4 من اللحم المفروم) تحتوى على ميكروب المكور العنقودى الذهبي وذلك بعد إجراء تفاعل البلمرة المتسلسل على 7 معزولات. أما بعد إجراء تفاعل البلمره المتعدد على هذه المعزولات للبحث عن جينات السموم المعوية وجدنا الأتي: أن البرجر يحتوى على عدد واحد جين من النوع seb بينما السجق يحتوى على عدد واحد جين من النوع sea أما بالنسبة للحم المفروم فقد وجد أنه يحتوى على عدد 2 جين من النوع sea وعدد واحد جين لكلا من sec, sed.

هذا وقد تم مناقشة النتائج والتوصيات اللازمة وذلك للحد من وجود هذا الميكروب فى منتجات اللحوم للحد من حالات التسمم الغذائي.