Detection of *Staphylococcus aureus* in some meat products using PCR technique

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A B S T R A C T

Staphylococcus aureus is frequently implicated in food borne illnesses. A total of 100 random samples of meat products represented by minced meat, beef burger, kofta and luncheon (25 of each) were collected from different supermarkets in Gharbia governorate. 25 grams from each sample were subjected to bacteriological examination for determination of Staphylococci count and *S. aureus* count, isolation and identification of *S. aureus* then molecular examination; using PCR technique as a confirmatory method for identification of *S. aureus* isolates. In addition, multiplex PCR was used for detection of classic enterotoxin genes (*sea*, *seb*, *sec* and *sed*) of *S. aureus* isolates. The mean values of Staphylococcal and *S. aureus* count /g were $3.41x10^3 \pm 0.58x10^3$ and $9.35x10^2 \pm 2.04x10^2$ for minced meat; $7.95x10^3 \pm 1.22x10^3$ and $1.87x10^3 \pm 0.36x10^3$ for beef burger; $2.12x10^4 \pm 0.48x10^4$ and $3.72x10^3 \pm 0.51x10^3$ for kofta and $9.06x10^2 \pm 2.15x10^2$ and $4.29x10^2 \pm 0.67x10^2$ for luncheon, respectively. *S. aureus* was detected in 36%; 52%; 64% and 12% of the examined samples of minced meat, beef burger, kofta and luncheon, respectively. The occurrence of enterotoxin genes was determined in 12 isolates (3 from each meat product). The incidence of *sea* was 2/3 (66.67%) in beef burger and 1/3 (33.33%) in kofta. The incidence of *seb* was 1/3 (33.33%) in luncheon only. The incidence of *sea* and *sed* was 1/3 (33.33%) in minced meat only. The incidence of *sea*, *seb* and *sec* was 1/3 (33.33%) in kofta only. Meat products are considered a good medium for the growth of Staphylococci and the production of toxins.

Keywords: *S. aureus*, meat products, enterotoxin, PCR.

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1. INTRODUCTION

In recent years, there has been a steady increase in the production and consumption of processed meat products worldwide because of their high nutritive value and convenience. However, processed meat products may constitute a public health hazard due to the possible presence of foodborne pathogenic bacteria which cause illness, intoxication and sometimes death (Rajic et al., 2007). Foodborne disease is a major public health problem and a common cause of illness and death worldwide (Kirk et al., 2014).

Among the increased demand of the meat products, it is important to make sure that these products are of good quality; they must be free from hazardous microorganisms or when present should be at a safe low level as the contamination of meat products leads to inferior quality and unfit for consumption. This reflected that workers were not bound to hygienic role of dressing clean clothes and regular washing of their hands during the different operations. This unhygienic personal conduct is a reflection of poor knowledge, practice and attitudes to meat handling (Mottin et al., 2011).

Staphylococcus aureus is recognized worldwide as an important food borne pathogen because of its ability to produce a wide range of extracellular toxin proteins and virulence factors typically resulting in sudden onset of nausea, vomiting and abdominal cramps (Unal and Cinar, 2012).
Food borne diseases can be prevented by destroying the bacteria through proper cooking. When *S. aureus* is allowed to grow in food it can produce toxins that cause illness. Although cooking destroys the bacteria, the toxin produced is heat stable (Nosier et al., 2015).

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

So, the current study was conducted to evaluate the bacteriological quality of some meat products in Gharbia governorate regarding total Staphylococcal and *S. aureus* counts as well as multiplex PCR for detection of classic enterotoxin genes (*sea*, *seb*, *sec* and *sed*) of *S. aureus* isolates.

2. MATERIAL AND METHODS

2.1. Collection of samples:

One hundred random samples of meat products represented by minced meat, beef burger, kofta and luncheon (25 of each) were collected from different supermarkets in Gharbia governorate, Egypt. All collected samples were separately kept in a sterile plastic bag and transferred in an ice box to the laboratory under complete aseptic conditions without undue delay and examined as quickly as possible. 25 grams from each sample were subjected to the bacteriological examination for detection of *S. aureus* in such products and application of PCR as confirmatory technique as well as characterization of its virulence factors.

2.2. Bacteriological examination:

2.2.1. Preparation of samples:

It was done according to (ICMSF, 1996).

2.2.2. Determination of total Staphylococci and S. aureus count (FDA, 2001):

One ml from each of prepared serial dilutions was spread over Baired Parker agar plate using a sterile bent glass spreader. The inoculated and control plates were inverted and incubated at 37°C for 48 hours. The developed colonies (shiny black colonies) were enumerated and total Staphylococcal count/g was calculated. The suspected colonies of *S. aureus* appear as black, shiny, circular, smooth and convex with narrow white margin and surrounded

by a clear zone extending into opaque medium were enumerated and *S. aureus* count /g was calculated.

2.2.3. Identification of Staphylococci species:

It was done by morphological examination (Cruickshank et al., 1975) then biochemical identification (MacFaddin, 2000) and examined for catalase activity test, oxidase test, growth at 10% NaCl, detection of Arginine decarboxylase (ADH), bile esculent test, mannitol test, detection of hemolysis, coagulase test, thermostable nuclease test "D-Nase activity" (Lachia et al., 1971) and fermentation of sugars.

2.3. Polymerase Chain Reaction (PCR):

Primer sequences of *S. aureus* used for PCR identification system were shown in table (1).

DNA extraction using QIA amp kit (Shah et al., 2009); amplification reaction for *nuc* gene (Chu et al., 2010) and amplification of enterotoxin genes (Mehrotra et al., 2000).

2.4. Statistical analysis:

The evaluation and interpretation of obtained results were carried out using of Analysis of Variance (ANOVA) test according to (Feldman et al., 2003).

Table (1): Primer sequences of *S. aureus* used for PCR identification system:

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
nuc (F)	**5′** GCGATTGATGGTGATACGGTT **′3**	270	Brakstad et al. (1992)
nuc (R)	**5′** AGCCAAGCCTTGACGAACTAAAGC **′3**		
sea (F)	**5′** TTGGAAACGGTTAAAACGAA**′3**	120	Rall et al. (2008)
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**		

3. RESULTS

3.1. Staphylococci count/g:

The results recorded in table (2) showed that the mean values of Staphylococcal count/g in the examined samples of minced meat, beef burger, kofta and luncheon were $3.41x10^3 \pm 0.58x10^3$; $7.95x10^3 \pm 1.22x10^3$; $2.12x10^4 \pm 0.48x10^4$ and $9.06x10^2 \pm 2.15x10^2$, respectively. According to ANOVA analysis in table (3), there were high significant differences ($P<0.01$) in total Staphylococcal count between the examined samples of meat products. According to Egyptian Organization for Standardization "EOS" (2005), table (4) showed that the accepted samples of meat products based on their Staphylococcal count /g were 9 (36%) in minced meat, 3 (12%) in beef burger, 0 (0%) in kofta and 14 (56%) in luncheon while unaccepted samples were 16 (64%) in minced meat, 22 (88%) in beef burger, 25 (100%) in kofta and 11 (44%) in luncheon.

3.2. Incidence and Staphylococcus aureus count/g:

The results recorded in table (5) showed that the mean values of *S. aureus* count/g in the examined samples of minced meat, beef burger, kofta and luncheon were $9.35x10^2 \pm 2.04x10^2$; $1.87x10^3 \pm 0.36x10^3$; $3.72x10^3 \pm 0.51x10^3$ and $4.29x10^2 \pm 0.67x10^2$, respectively. Moreover, the results recorded in the same table revealed that the incidence of *S. aureus* in the examined samples of meat products was 9 (36%) in minced meat; 13 (52%) in beef burger; 16 (64%) in kofta and 3 (12%) in luncheon. According to Egyptian Organization for Standardization "EOS" (2005), all positive samples of such meat products were considered unaccepted as permissible limit of EOS for *S. aureus* must be free. According to ANOVA analysis in table (6), there were high significant differences ($P<0.01$) in *S. aureus* count between the examined samples of meat products.

3.3. Incidence of Staphylococcus species:

The results achieved in table (7) showed the incidence of *Staphylococcus* species isolated from the examined minced meat, beef burger, kofta and luncheon. The incidence of *Staphylococcus aureus* was 9 (36%); 13 (52%); 16 (64%) and 3 (12%), respectively. The incidence of *Staphylococcus epidermidis* was 6 (24%); 3 (12%); 5 (20%) and 2 (8%), respectively. The incidence of *Staphylococcus intermedius* was 0 (0%); 1 (4%); 1 (4%) and 0 (0%), respectively. The incidence of *Staphylococcus saprophyticus* was 2 (8%); 0 (0%); 3 (12%) and 0 (0%), respectively. The incidence of *Staphylococcus xylosus* was 1 (4%) in kofta only.

3.4. PCR results:

Photograph (1) showed that agarose gel electrophoresis of PCR of *nuc* gene (270bp) which is specific for demonstration and characterization of *S. aureus*. Lanes from 1 to 12 are positive *S. aureus* strains for *nuc* gene. The results recorded in table (8) and photograph (2) showed that, by applying multiplex PCR technique, the incidence of enterotoxin genes of *S. aureus* isolated from the examined minced meat, beef burger, kofta and luncheon was as following: The incidence of *sea* was 2 (66.67%) in beef burger and 1 (33.33%) in kofta. The incidence of *seb* was 1 (33.33%) in luncheon only. The incidence of *sea* and *sed* was 1 (33.33%) in minced meat only. The incidence of *sea*, *seb* and *sec* was 1 (33.33%) in kofta only. The incidence of negative strains was 2 (66.67%); 1 (33.33%); 1 (33.33%) and 2 (66.67%), respectively.

Table (2): Total Staphylococcal count/g in the examined samples of meat products (n=25).

Meat Products	Min	Max	Mean ± S.E*
Minced meat	1.0×10^2	9.0×10^3	$3.41\times10^3 \pm 0.58\times10^3$
Beef burger	1.0×10^2	2.2×10^4	$7.95\times10^3 \pm 1.22\times10^3$
Kofta	1.1×10^3	6.0×10^4	$2.12\times10^4 \pm 0.48\times10^4$
Luncheon	1.0×10^2	5.0×10^3	$9.06\times10^2 \pm 2.15\times10^2$

S.E* = Standard error of mean

Table (3): Analysis of variance (ANOVA) of total Staphylococcal count in the examined samples of meat products.

Source of variance	D.F	S.S	M.S	F.value
Total	99	73992.27		
Between Products (T)	3	33826.83	11275.61	26.95^{++}
Error	96	40165.44	418.39	

D.F = Degrees of freedom, M.S = Mean squares, S.S = Sum squares
++ = High significant differences (P<0.01)

Table (4): Acceptability of the examined samples of meat products based on their Staphylococcal counts/g (n=25).

Products	Staphylococci count /g*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Minced meat	$> 10^2$	9	36	16	64
Beef burger	$> 10^2$	3	12	22	88
Kofta	$> 10^2$	--	--	25	100
Luncheon	$> 10^2$	14	56	11	44

*Egyptian Organization for Standardization "EOS" (2005), The accepted limits must be not exceed 10^2 cfu/g, No 1694-2005 for minced meat, No 1688-2005 for beef burger, No 1973-2005 for kofta, No 1114-2005 for luncheon.

Table (5): Incidence and *Staphylococcus aureus* count/g in the examined samples of meat products (n=25).

Meat Products	+ve samples		Min	Max	Mean ± S.E*
	No.	%			
Minced meat	9	36	1.0×10^2	2.0×10^3	$9.35\times10^2 \pm 2.04\times10^2$
Beef burger	13	52	1.0×10^2	5.4×10^3	$1.87\times10^3 \pm 0.36\times10^3$
Kofta	16	64	1.0×10^2	1.7×10^4	$3.72\times10^3 \pm 0.51\times10^3$
Luncheon	3	12	1.0×10^2	9.0×10^2	$4.29\times10^2 \pm 0.67\times10^2$

S.E* = Standard error of mean, N.B. All positive samples of such meat products were considered unaccepted according to EOS (2005) (Permissible limit of EOS for *S. aureus* must be free).

Table (6): Analysis of variance (ANOVA) of *Staphylococcus aureus* count in the examined samples of meat products.

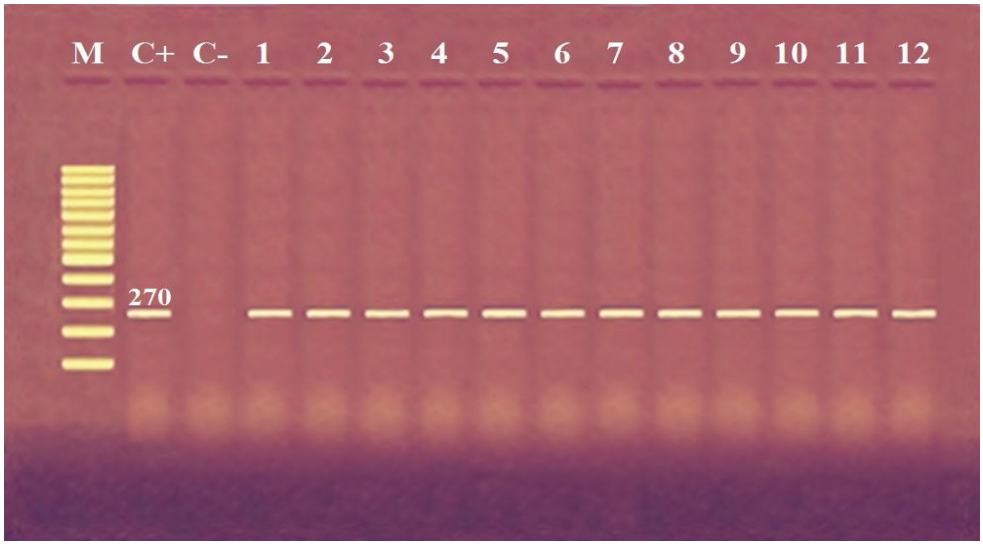
Source of variance	D.F	S.S	M.S	F.value
Total	99	33097.54		
Between Products (T)	3	9603.45	3201.15	13.08^{++}
Error	96	23494.09	244.73	

D.F = Degrees of freedom, M.S = Mean squares, S.S = Sum squares,
++ = High significant differences (P<0.01)

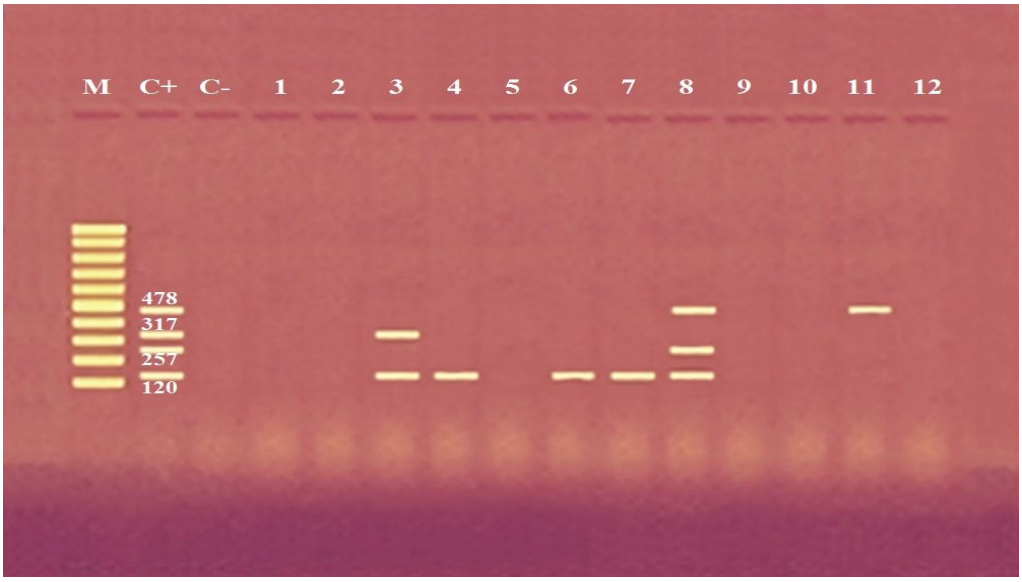
Table (7): Incidence of Staphylococcus species isolated from the examined samples of meat products (n=25).

Staphylococcus species	Meat products									
	Minced meat		Beef burger		Kofta		Luncheon		Total (n=100)	
	No.	%	No.	%	No.	%	No.	%	No.	%
S. aureus	9	36	13	52	16	64	3	12	41	41
S. epidermidis	6	24	3	12	5	20	2	8	16	16
S. intermedius	--	--	1	4	1	4	--	--	2	2
S. saprophyticus	2	8	--	--	3	12	--	--	5	5
S. xylosus	--	--	--	--	1	4	--	--	1	1

N.B. The isolation % was calculated according to the total number of samples.



Photograph (1): Agarose gel electrophoresis of PCR of *nuc* gene (270 bp) specific for demonstration and characterization of *S. aureus*.
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive *S. aureus* for *nuc* gene.
Lane C-: Control negative *S. aureus*.
Lanes from 1 to 12: Positive *S. aureus* strains for *nuc* gene.



Photograph (2): Agarose gel electrophoresis of multiplex PCR of *sea* (120 bp), *seb* (478 bp), *sec* (257 bp), and *sed* (317 bp) enterotoxin genes for characterization of *S. aureus*.
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive for for *sea*, *sec*, *sed* and *seb* genes.
Lane C-: Control negative.
Lanes 4, 6 and 7: Positive *S. aureus* strains for *sea* gene.
Lane 11: Positive *S. aureus* strain for *seb* gene.
Lane 3: Positive *S. aureus* strain for *sea* and *sed* genes.
Lane 8: Positive *S. aureus* strain for *sea*, *seb* and *sec* genes.
Lanes 1, 2, 5, 9, 10 and 12: Negative *S.aureus* strains for enterotoxins.

Table (7): Incidence of enterotoxin genes of *S. aureus* isolated from the examined samples of meat products by using multiplex PCR.

Enterotoxin	Minced meat (3)		Beef burger (3)		Kofta (3)		Luncheon (3)		Total (12)	
	No.	%	No.	%	No.	%	No.	%	No.	%
A	--	--	2	66.67	1	33.33	0	0	3	25
B	--	--	--	--	--	--	1	33.33	1	8.33
A and D	1	33.33	--	--	--	--	--	--	1	8.33
A, B and C	--	--	--	--	1	33.33	--	--	1	8.33
-ve strains	2	66.67	1	33.33	1	33.33	2	66.67	6	50
Total	3	100	3	100	3	100	3	100	12	100

4. DISCUSSION

Staphylococci exist in air, dust, equipment, food, environmental surfaces, humans and /or animals. Staphylococci usually present in the nasal passage, throat, on the hair and skin (FDA, 2012). There are many types of Staphylococci, but most infections are caused by *S. aureus* which is the third most common cause of food poisoning in the world (Acco et al., 2003).

The results recorded in table (2) were nearly similar to Lela-Radwa (2016) (2.63 $x10^3$ cfu/g in minced meat); Abd EL-Fatah (2015) ($1.57x10^3$ cfu/g in beef burger) and Heweidy (2017) ($1.68x10^2$ cfu/g in luncheon). Higher results were recorded by Elmali and Yaman (2005) ($1.0x10^5$ cfu/g in kofta) and Ibrahim-Shimaa (2016) ($5.83x10^5$ cfu/g in minced meat; $2.08x10^5$ cfu/g in beef burger and $3.00x10^7$ cfu/g in luncheon). While lower results were detected by Elmali and Yaman (2005) ($2.2x10^2$ cfu/g in minced meat) and Lela-Radwa (2016) ($6.61x10^2$ cfu/g in kofta).

The high count of Staphylococci in meat samples indicates the presence of cross contamination which is usually related to human skin, hand touch and discharge from human (Postgate, 2000).

The results recorded in table (5) showed that the mean values of *S. aureus* count/g were nearly similar to those reported by Elmali and Yaman (2005) ($6.3x10^3$ cfu/g in kofta); EL-Daly et al. (2014) ($6.25x10^3$ cfu/g in beef burger) and Lela-Radwa (2016) (3.16 $x10^2$ cfu/g in luncheon and $1.51x10^2$ cfu/g in minced meat). Higher results were recorded by Tarabees et al. (2016) ($2.98x10^5$ cfu/g in minced meat; $5.73x10^4$ cfu/g in beef burger and $3.09x10^5$ cfu/g in luncheon). While lower results were reported by Elmali and Yaman (2005) (1.6x10 cfu/g in minced meat); Lela-Radwa (2016) (2.82 $x10^2$ cfu/g in kofta) and EL-Shabacy- Rasha (2017) (4.17 $x10^2$ cfu/g in beef burger).

Moreover, the results recorded in table (5) revealed that the incidence of *S. aureus* in the examined samples of meat products was nearly similar to Shawish and AL-Humam (2016) (38% in minced meat). Higher results were recorded by Mohammed (2010) (80% in beef burger and kofta); Mousa et al. (2014) (80% in luncheon and 68% in beef burger) and Tarabees et al. (2016) (70% in minced meat). While lower results were detected by Awadallah- Maysa et al. (2014) (10% in luncheon); Shawish and AL-Humam (2016) (22% in beef burger) and Nadim (2016) (28% in minced meat and 36% in kofta).

It was clear from the above results that the lowest contamination was in luncheon and this may be due to food borne diseases can be prevented by destroying the bacteria through cooking. When *S. aureus* is allowed to grow in food it can produce toxins that cause illness. Although cooking destroys the bacteria, the toxin produced is heat stable (Wagner, 2008). The contamination here may be attributed to the workers who can transfer Staphylococci on their hands to equipment and to the product during manipulation. Luncheon recontamination during slicing can be a concern at both industry and retail level (Mottin et al., 2011). Also, it was clear that the highest contamination was in kofta and beef burger. Meat products are subjected to contamination with several types of pathogenic microorganisms from different sources during preparation, processing and

serving to consumers. This varies according to the method of manufacture, quality of used non-meat ingredient and contamination level during the processing chain, packaging and storage (Borch and Arinda, 2002).

Development of identification techniques for a clinical rapid diagnosis is necessary. PCR is a rapid, sensitive, and less time-consuming than the conventional bacteriological identification methods (Chiang et al., 2006) and extensively used to identify bacteria isolated from different kind of samples, including foods (Eijakee et al., 2013). Several pathogens can be detected simultaneously in one step by using multiplex PCR technique with special concern to enterotoxigenic strains of *S. aureus* (Ngamwongsatit et al., 2008).

The results recorded in table (8) and photograph (2) showed that *sea* was the predominant gene in the examined samples of meat products. Although several Staphylococcal enterotoxins (SEs) have been identified, *sea* is a highly heat stable SE and is the most common cause of SFP worldwide. Symptoms of SFP include nausea, vomiting and abdominal cramps with or without diarrhea (Kadariya et al., 2014).

From the above results it can be concluded that meat products are considered a good medium for the growth of Staphylococci and the production of toxins. The lowest contamination was in luncheon but the highest contamination was in kofta and beef burger. The presence of this bacterial species in the final retail products is a result of contamination during the manufacturing, distribution, storage, slicing, packaging, and retail sale of the products. This subsequently contributes to health risks to the consumer. All precautions of proper sanitation during manufacture, handling and storage of such meat products should be carried out to control these serious pathogens and to obtain a maximum limit of safety to consumers.

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