Detection of *Staphylococcus aureus* and enterotoxin genes from meat products by using conventional and modern identification methods

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A B S T R A C T

One hundred meat product samples (minced meat, kofta, burger and sausage, 25 of each) were collected and examined for bacteriological evaluation and detection of *Staphylococcus aureus* by conventional bacteriological methods and by polymerase chain reaction PCR. The mean value of *S. aureus* counts cfu/g were 0.63×10^2 $\pm0.19\times10$, $0.17\times10^2\pm$, $0.96\times10^2\pm0.06\times10^2$ and $1.1\times10^2\pm0.15\times10^2$ respectively. Concerning *S.aureus*, bacteriological results revealed that the prevalence in minced meat, kofta, burger and sausage was 16%, 12%, 16%, 20%, respectively. In addition, 5 samples out of 100 ones were unaccepted as they were exceeded the permissible limit of E.O.S 2005. Moreover, four random positive and negative *S. aureus* samples were reexamined by polymerase chain reaction (PCR) and just one sample was negative by the bacteriological method for *S. aureus* showed positive results with PCR. Multiplex Polymerase Chain Reaction (m-PCR) was applied for detection of genes responsible for enterotoxins production (sea, seb, sec, sed and see) from identified coagulase positive *Staphylococcus aureus*. They were detected in the examined minced meat and sausage samples.

Keywords: *Meat products; S. aureus; clfA; enterotoxin; m-PCR.*

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1. INTRODUCTION

The increasing number and severity of food poisoning outbreaks worldwide have considerably increased public awareness concerning food (Forsythe, 2008), particularly meat and meat products which are one of the most important sources of human infections with different foodborne pathogens (Norrung, *et al.,* 2009). However, meat and meat products continue to be an important food group in the diet for many consumers (Speedy, 2003). *Staphylococcus aureus* is one of the most pathogenic species and it is responsible for the third of food-borne illness in the world (Normanno *et al*., 2007). When staphylococci contaminate food, they produce some enzymes which are implicated with the ability of invasiveness and some of extracellular substances are heat stable enterotoxins. Extensive cooking can kill the bacteria however the toxins may not be destroyed as a results of most of them are gene-based i.e. they can be carried on the plasmid (Prescott *et al.*, 2005). The Staphylococcal enterotoxins (SEs) are responsible for the

symptoms associated with Staphylococcal food poisoning (Llewelyn and Cohen, 2002).

Microbiological assessment is important to determine the safety and quality of food. In the past isolation and identification of microorganisms were depend on cultural techniques. These methods are the most reliable and accurate in the detection of foodborne pathogens. However, they are labor intensive, have long processing times and are costly. The major disadvantage to the current methodology is that it can take 2- 3 days for any results to show up and up to ten days for confirmation (Jasson *et al.,* 2010). Multiplex polymerase chain reaction m-PCR is a powerful technique and has application in the detection of pathogens in food samples. m-PCR is an advantage against the culturing methods as you will be able to use different amounts of selective DNA in one PCR reaction. Recent reports have shown that m-PCR greatly improves specificity and sensitivity for the detection of pathogens (Huang *et al.,* 2009).

The multiplex PCR assay for detection of Staphylococcal enterotoxins genes (*SEA*, *SEB*, *SEC*, *SED* and *SEE*) was developed and proved to be a specific, sensitive, and rapid method. (Zschock *et al.,* 2005). Therefore the current study was planned out throw light on the count and also the incidence of *S. aureus* followed by PCR confirmation and m-PCR identification of enterotoxigenic strains in meat products (minced meat; kofta; burger and sausage) sold in supermarkets at Kaliobia Governorate.

Materials and methods

2.1. Collection of samples:

A total of 100 random samples of meat products; minced meat; beef kofta; beef burger and sausage (25 for each), were purchased from different supermarkets at Kaliobia Governorate, Samples were submitted to the lab in Animal Health Research Institute for bacteriological examination to detect the incidence of *Staphylococcus aureus* and its enterotoxin genes by using conventional and modern identification methods and evaluate the hygienic health hazard of them with Staphylococci.

2.2. Bacteriological examination:

2.2.1. Preparation of samples (APHA, 2001)

2.2.2. Determination of Staphylococci and S. aureus counts (FDA, 2001).

*2.2.3. Isolation and identification of suspected S. aureus: a*ccording to Quinn et al (2002), the samples were cultured for isolation of S. aureus onto peptone water for 24 hours at 37ºC and then a loopful was taken and cultured onto mannitol salt agar and then onto Baird parker medium. All inoculated plates were incubated at 37ºC for 24-48 hours then colonies were identified. The colonies characterized by circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet-black, frequently with light-colored (offwhite) margin, surrounded by opaque zone and frequently with an outer clear zone. All suspected colonies are tested and confirmed biochemically and coagulase activities.

2.3. Polymerase Chain Reaction (PCR):

Using polymerase chain reaction (PCR) in 4 random sample for Polymerase Chain Reaction (PCR) of 4 random S. aureus meat products samples two positive (minced meat- sausage) and two negatives (burger- kofta).

2.4. Multiplex polymerase chain reaction (m-PCR): for detection of classic enterotoxin genes (A, B, C, D and E) of positive S. aureus meat products samples (minced meat, burger and kofta) one sample of each.

DNA extraction and purification direct from the meat products samples, following QIAamp DNA Mini Kit (Catalogue no.51304), Emerald Amp GT PCR master mix (Takara) with Code No. RR310A, 1.5% agarose gel electrophoreses (Sambrook et al., 1989) using the Primers sequence, target genes amplicons sizes and cycling conditions showed in Tables (1) and (2).

2.5. Statistical analysis:

Data obtained were analyzed according to Snedecor and Cochran (1969) using the computer software program (SPSS, 2001).

3. RESULTS

The results of the examined meat products (minced meat, kofta, burger and sausage) are presented in Tables (1-4) and Figures (1-2).

The results of bacteriological examination of meat products revealed that Staphylococcus counts were highest in minced meat then burger and kofta then sausage. While, *Staphylococcal aureus* count was highest in sausage then burger and minced meat then kofta, in which the incidence of co-agulase positive *Staph. aureus* is highest in sausage then burger and minced meat then kofta.

The results of molecular identification showed that three out of Four random sample (one of each product) (two positive and two negative) were positive for *S. aureus by using uniplex PCR*. In addition, the three positive *S. aureus samples* were reexamined by m-PCR for detection of different enterotoxins and the results revealed that one isolate carrying *Seb* gene, and other carrying *Sed* gene, while one negative for all enterotoxins.

Table1: Cycling conditions of *S.aureus* primers during cPCR

Target	Cyclic condition	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
S. aureus	For detection of *S. aureus*	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
	For detection of enterotoxins	94˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

Table 2: A detailed descriptions of the designed oligonucleotide primers sequences used

Target	Primer	Gene	Sequence (5' -3')	Amplified product	Reference
S. aureus	F	*clfA*	GCAAAATCCAGCACAACAGGAAACGA	638 bp	Mason *et al.*, 2001
	R		CTTGATCTCCAGCCATAATTGGTGG		
	F	*Sea*	GGTTATCAATGTGCGGGTGG	102 bp	Mehrotra *et al.*, 2000
	R		CGGCACTTTTTTCTCTTCGG		
	F	*Seb*	GTATGGTGGTGTAACTGAGC	164 bp	
	R		CCAAATAGTGACGAGTTAGG		
	F	*Sec*	AGATGAAGTAGTTGATGTGTATGG	451 bp	
	R		CACACTTTTAGAATCAACCG		
	F	*Sed*	CCAATAATAGGAGAAAATAAAAG	278 bp	
	R		ATTGGTATTTTTTTTCGTTC		
	F	*See*	AGGTTTTTTCACAGGTCATCC	209 bp	
	R		CTTTTTTTTCTTCGGTCAATC		

Table 3: Total Staphylococci counts/g in the examined samples of meat products (n=25)

Samples	Positive		Min.	Max.	Mean ±SEM***
	No.	%			
Minced Meat	25	100	0.3×10^2	3.1×10^3	$1.2\times10^3 \pm1.8\times10^{2a}$
Kofta	25	100	1.0×10^2	5.3×10^3	$1.06\times10^3\pm2.2\times10^{2a}$
Burger	24	96	0.1×10^2	2.8×10^3	$1.09\times10^3 \pm1.7\times10^{2a}$
Sausage	25	100	0.1×10^2	2.8×10^3	$0.97\times10^3 \pm1.8\times10^{2a}$

N.B.:- There was no sig. difference between different meat products.

Table 4: Staphylococcus *aureus* counts/gm. in the examined samples of meat products (n=25)

Samples	Min.	Max.	Mean ±SEM
Minced Meat	0.33×10^2	1.2×10^2	$0.63\times10^2\pm0.19\times10^{2ab}$
Kofta	0.05×10^2	0.25×10^2	$0.17\times10^2 \pm0.06\times10^{2b}$
Burger	0.5×10^2	1.6×10^2	$0.96\times10^2\pm0.24\times10^{2a}$
Sausage	0.72×10^2	1.5×10^2	$1.1\times10^2\pm0.15\times10^{2a}$

N.B. Different letter within the same column indicates sig. difference.

Table 5: Acceptability of Total *S. aureus* counts in the examined samples of meat products (n=25)

Samples	PL*	Accepted samples		Unaccepted samples	
		No.	%*	No.	%*
Minced Meat	10^2	24	96	1	4
Kofta	10^2	25	100	0	0
Burger	10^2	23	92	2	8
Sausage	10^2	23	92	2	8

*permissible limits according to EOS (2005).

Table 6: Incidence of *S.aureus* in the examined meat products samples (n=25)

Samples	Positive *S.aureus* samples	
	No.	%
Minced Meat	4	16
Kofta	3	12
Burger	4	16
Sausage	5	20
Total	16	16

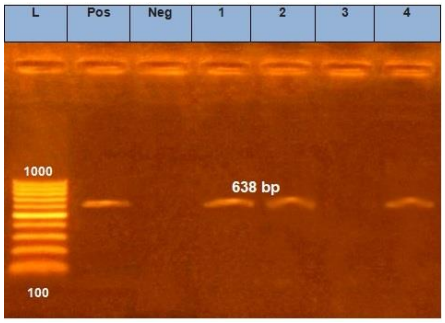

Fig.1. Agarose gel electrophoresis showing PCR products of clfA gene (638bp) specific for characterization of *S.aureus*. Lane L: 100-1000 bp DNA Ladder. Neg.: Negative control (Salmonella reference: ATCC14028). Pos.: Positive control (*S. aureus* reference: ATCC25923 at 638 bp). Lane 1; 2&4: Positive *S. aureus* (minced meat, sausage and burger) one of each.Lane 3: Negative *S. aureus*.

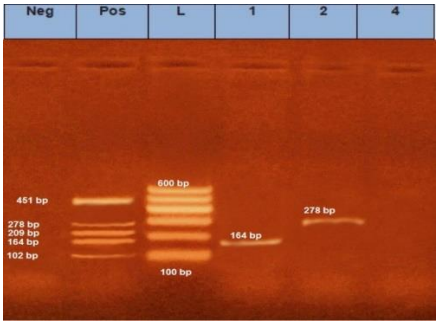

Fig.2. Agarose gel electrophoresis of multiplex PCR of *sea* (102), *seb* (164), *sec* (451), *sed* (279) and *see* (209) enterotoxin genes for characterization of *s.aureus* isolated from Minced meat, Burger and sausage (one from each product). Lane L: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control for *sea*, *seb*, *sec*, *sed* and *see*. Lane 1: Positive *S. aureus* strain for *seb* gene (minced meat). Lane 2: Positive *S. aureus* strain for *sed* gene (sausage). Lane 4: Negative *S. aureus* strain for enterotoxins (burger).

4. DISCUSSION

The foodborne pathogens are able to cause serious public health problems, particularly in developing countries where they cause a high level of morbidity and mortality rates. Quick, sensitive, specific and simple techniques for detection of the foodborne pathogens are required for the effective implementation of food safety. Polymerase chain reaction (PCR) has become an indispensable tool in molecular diagnostics and can be very efficiently used in rapid detection of food-borne pathogens (Pinto *et al.,* 2005).

4.1. Total Staphylococcus count:

Staphylococci were normally inhabitant in animal and man, as a results of their ubiquitous occurrence in nature, they were found in numerous raw foods, at the meanwhile foodborne illness from Staphylococcus enterotoxins remains a major problem worldwide (Normanno *et al*., 2005). Staphylococcus counts (cfu/g) of the examined meat products (minced meat; kofta; burger and sausage) came in agreement with those reported by Abd El-Fatah-Rabab (2015). Meanwhile, a lower results were recorded by Karaboz and Dincer (2002) and Gibriel *et al*. (2007).

Moreover, the statistical results revealed that there was no significant difference (P>0.05) of Staphylococci counts between meat product samples (minced meat, beef kofta, burger and sausage. These results came in accordance

with that obtained by Maarouf and Nassif-Marionette (2008) and Abd El- Salam-Azza *et al.* (2014).

4.2. Staphylococcus aureus count:

The presence of *S. aureus* in foods commonly indicates direct contamination from worker's hands with abrasion and wounds or inadequately cleaned equipment resulting in *S. aureus* intoxication. Accordingly, the total *S. aureus* count can be taken as an indicator of sanitary degree under which the meat and its products are processed and handled (potter, 2001). The recorded results in (Table, 4) revealed that, mean values of the examined meat products (minced meat; beef kofta; beef burger and sausage) samples were $0.63\times10^{2}\pm0.19\times10^{2ab}$; $0.16\times10^{2}\pm0.06\times10^{2b}$; $0.96\times10^{2}\pm0.24\times10^{2a}$ and $1.1\times10^{2}\pm0.15\times10^{2a}$, respectively. These results disagreed with those of Oluwafemi and Simisaye (2006) Gibriel *et al*. (2007) who recorded higher *S. aureus* counts in examined samples.

Results obtained in the table (5) revealed that 4%, 0%, 8% and 8%, respectively of minced meat, kofta, beef burger and sausage were exceeded the permissible limit according to E.O.S. (2005). So that the samples which exceeding the permissible limit represent a potential health risk at which under favorable condition, *S. aureus* can proliferate and produce enterotoxin causing SFP.

4.3. Isolation of Staphylococcus aureus

As shown in Table (6), the percentages of *S. aureus* isolated from the examined minced meat, kofta; burger and sausage samples were 16%, 12%, 16%, 20%, respectively.

The obtained results of *S. aureus* in the examined samples were nearly similar to those of Omar *et al.,* (2009) and Soultos *et al.* (2003), and lower than those obtained by Vorster *et al.* (1994) and El-Khateib, (1997).

In general contamination with *S.aureus* may occur during the preparation, packaging, transportation and storage of meat products in supermarkets or directly from infected food-producing animals. However the low percentage of *S. aureus* in kofta and burger may be due to the addition of spices during manufacture and good hygiene.

4.4. Polymerase Chain Reaction (PCR).

PCR offers sensitive and specific detection of pathogens. Within the last ten years, several authors have proposed the use of m-PCR for the detection of foodborne pathogens to replace the conventional methods. They are rapid, easy to handle, sensitive and specific and therefore constitute very valuable tools for routine applications. This study showed that the PCR technique was very convenient to take DNA templates directly from the meat products samples after DNA extraction and there is no need to take from the culture as it time-consuming, labour intensive and very costly as reported by Chen *et al.,* (2012) and Kim *et al.,* (2014) who examined directly from food samples without the use of bacterial cultures but with different primers used in this study unlike Latha *et al.,* (2014) who examined their PCR technique by the use of bacterial culture.

Four random *S. aureus* samples (two positives and two negatives) by the conventional method, were reexamined by PCR, there was great agreement between results of conventional method for and PCR technique in three random samples (2 positive and 1 negative), while one sample was negative by conventional method for S. aureus, showed positive results with PCR (false negative) as in fig. (1).

This result clarified the high sensitivity of the PCR technique in detection the false negative results of the traditional microbiological culture method. Similar results were obtained

by Chen *et al.*, (2012) who detected a false negative result (negative by conventional method and positive by m-PCR.

The false negative result may due to the low number of bacterial load which can't be detected by microbiological assay. Also, inhibition of some microbes to selective microbe appearance on the media. So the m-PCR assay has the potential to be used in routine diagnostic laboratories and also as a rapid screening tool in food testing laboratories to identify food samples quickly especially in case of outbreaks.

4.5. Multiplex PCR for detection of classic enterotoxin.

It was clear that 3 isolates of examined minced meat, burger and sausage samples (one of each), were subjected to multiplex PCR for detection of *S. aureus* classic enterotoxin genes and the results were as follow: isolate for minced meat sample mostly harbored (1) *seb*. Meanwhile, isolate for sausage sample harbored (1) *sed* gene and the isolate of burger sausage sample was negative for all classic enterotoxin genes fig. (2).

PCR is rapid and specific method for detection of different food borne pathogen in meat products samples. It gives the ability to detect bacteria cells within a little time and PCR was demonstrated to be accurate methods for identification.

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