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Molecular detection of enterotoxigenic *Staphylococcus aureus* in ready-to-eat beef products

Marwa Saif[1], Saad M. Saad[2], Faten Hasanine[2], Fahim Shaltout[2], Marionette Zaghloul[1]

[1]*Department of Food Hygiene, Animal Health Research Institute, Benha branch, Egypt*

[2]*Department of Food Hygiene, Faculty of Veterinary Medicine, Benha University, Egypt*

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ABSTRACT

Ready to eat (RTE) meat products are considered a rapid way to animal protein intake which may be preferred by consumers due to its palatability and quality; unfortunately, cooked meat products may be loaded by many foodborne pathogens especially *Staphylococcus aureus* (*S. aureus*) due to post cooking cross-contamination through mishandling and/or getting in touch with raw materials; so, this study aimed to investigate the incidence of *S. aureus* in some RTE meat products followed by molecular detection of enterotoxigenic *S. aureus* isolates and its enterotoxin genes in 120 RTE meat products represented by luncheon, burger, shawerma and kofta (30 of each) which were collected from different restaurants and street vendors in Benha city, Qalubiya governorate, Egypt. Results revealed that kofta samples were the most contaminated samples with mean values 3.42×10^3, and 5.2×10 CFU/g for total staphylococci and *S. aureus* counts, respectively; followed by burger, shawerma and luncheon samples. Multiplex PCR detection of *S. aureus* enterotoxins (SE) genes revealed detection of different isolates carrying staphylococcal enterotoxin type A, type B, mixed strain carrying both staphylococcal enterotoxins type A and type D. So, RTE meat products of low hygienic measures may be risky to consumer's health.

1. INTRODUCTION

Meat and meat products are the most palatable of highly nutritious and highly desirable foods for human-being, as they are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients (Zafar*et al.*, 2016). Staphylococcal foodborne disease is caused by contamination of food during preparation or serving by performed *S. aureus* enterotoxin (Murray, 2005).

Staphylococcus aureus is a cluster forming spherical Gram-positive bacterium which is known to cause foodborne intoxication, as some of its pathogenic strains are capable of producing heat-stable enterotoxins. Moreover, it is a facultative anaerobic bacterium possesses a wide spectrum of virulence properties, including extracellular proteins like adhesions, invasions, hemolysins, exotoxins; staphylococcal enterotoxins are recognized as the most virulent factors due to its pathogenicity. The production of staphylococcal entererotoxins (SEs) by this bacterium is recognized as one of the predominant foodborne problems causing gastroenteritis worldwide; contamination by toxigenic *S. aureus* in RTE food is a major public health issue in both developing countries like Vietnam, and developed countries like the USA, Japan; during 1997, approximately 185,000 people suffered from the SE related food-poisoning including thousands of deaths (Mead et al., 1999).

Staphylococcus aureus is considered the third-most important cause of food-borne disease in the world (Normanno et al., 2007) due to having two aggravating characteristics: toxin production, and wide range of antimicrobial resistance. This pathogen is considered an excellent indicator of thermal processing inefficiency, inadequate hygienic conditions during food production/preparation, or improper cooling after food preparation (Jay, 2000).

Staphylococcal food poisoning (SFP) is a mild intoxication occurring after the ingestion of food containing from 20ng to <1µg of staphylococcal enterotoxin, enough to determine symptoms in human beings. *Staphylococcus aureus* produces a spectrum of extracellular protein toxins and virulence factors which are thought to contribute to the pathogenicity of the organism.

* Corresponding author **Marwa Saif.** Department of Food Hygiene, Animal Health Research Institute, Benha branch, Egypt

staphylococcal enterotoxins (SEs) are serologically grouped into five major classical types which are SEA, SEB, SEC, SED and SEE in addition to toxic shock syndrome toxin (TSST-1) which causes toxic shock syndrome in human (Chiang et al., 2006).

Enterotoxin, of types A, B, C, D, and E, are known to be thermostable and responsible for the clinical presentation of SFP; the certain mode of their action is not clear, but it is known to accelerate the intestinal peristalsis; consumption of contaminated foods with these enterotoxins, results in diarrhea, nausea, abdominal cramps, and a characteristic projectile vomiting within next 2-8 hours depending on the individual susceptibility and toxic dose ingested. Detection of staphylococcal enterotoxins in foods can be proven by immunodiagnostic methods as well as molecular biology techniques like the Polymerase Chain Reaction (PCR) (Edwin, 1989; Le Loir et al., 2003).

Therefore, this study aimed to investigate the incidence, and molecular detection of *S. aureus* and the enterotoxigenic strains carrying enterotoxin genes in some RTE meat products served in restaurants and street vendors in Benha city, Qalubiya governorate, Egypt.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A grand total of 120 samples of RTE beef products represented by "luncheon, burger, shawerma and kofta" (30 of each) were collected from different restaurants and street vendors in Benha city, Qalubiya governorate, Egypt. Samples were transferred to the laboratory under complete aseptic conditions in ice box within one hour and examined for bacteriological and molecular detection of the incidence of toxigenic S. aureus contamination.

2.2. Bacteriological examination:

- Preparation of sample according to APHA (2013).
- Determination of *Staphylococci and S. aureus* count according to (ISO 6881-1:1999, A1:2003).
- Identification of *Staphylococcus aureus*.
- Morphological examination (Cruickshank et al., 1975).
- Biochemical identification (MacFaddin, 2000).

2.3. Molecular detection of S. aureus enterotoxin genes:

Two isolates of each confirmed coagulase-positive S. aureus strains from each examined product were sent to the Central Laboratory for Food Analysis, Faculty of Veterinary Medicine, Benha University, Egypt; and molecularly examined for the presence of S. aureus enterotoxins types A through D using multiplex PCR.

- Primer sequences of S. aureus used for PCR system following Rall et al. (2008) as mentioned in table (1).
- DNA Extraction using QIA amp kit (Shahet al., 2009).
- Amplification of S. aureus enterotoxin genes (Rall et al., 2008).

Table 1 Primer sequences of S. aureus used for PCR system

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Ref.
sea (F)	5′ TTGGAAACGGTTAAAACGAA'3	120	Rall et
sea (R)	5′ GAACCTTCCCATCAAAAACA '3		al. (2008)
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		

2.4. Statistical Analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

Results of total staphylococcal counts in the examined samples were summarized in table (2), where kofta was the most contaminated samples followed by burger, shawerma, and luncheon samples; staphylococci were detected in 67.5% of all examined samples, represented by 73.3, 63.3, 50.0, and 83.3% with mean values 3.42×10^3, 2.81×10^3, 1.53×10^3, and 0.20×10^3CFU/g for kofta, burger, shawerma, and luncheon, respectively; statistical analysis of variance indicated that no significant difference was detected between both kofta and burger samples, while significant decrease in total staphylococcal counts was detected between them and the other samples when $P \leq 0.05$.

Table 2 Statistical analytical results of staphylococci in examined ready to eat beef products (n=30).

Product	+ ve samples		Counts (CFU\g)		
~~Product~~	No.	%*	Min.	Max.	Mean ± SE
kofta	22	73.3	1.0×10^3	5.3×10^3	$3.42\times10^3\pm 0.60\times10^{3a}$
Burger	19	63.3	1.7×10^3	5.0×10^3	$2.81\times10^3\times 0.37\times10^{3a}$
Shawerma	15	50.0	1.1×10^3	1.9×10^3	$1.53\times10^3\pm 0.09\times10^{3b}$
Luncheon	25	83.3	0.19×10^3	0.23×10^3	$0.20\times10^3\pm 0.01\times10^{3c}$
Total	81	67.5**			

Values within a column with different superscript letters were significantly different at ($P \leq 0.05$). * Percentage in relation to total number of each sample (30). ** Percentage in relation to total number of samples (120).

In addition, results of the incidence and counts of S. aureus in the examined samples as demonstrated in the table (3) revealed detection of S. aureus in 50.8% of all examined samples, where kofta was also the most contaminated samples with S. aureus followed

by burger, shawerma, and luncheon with mean values of 5.2×10, 3.2×10, 2.6×10 and 1.9×10 CFU/g, with incidences of 56.6, 43.3, 36.6, and 66.6%, respectively; statistical analysis of variance indicated a significant difference between kofta and the other samples when $P \leq 0.05$.

Table 3 Statistical analytical results of Staphylococcus aureus in examined ready to eat beef products (n=30).

Product	+ ve samples		Count (CFU\g)		
Product	No.	%*	Min.	Max.	Mean ± SE
kofta	17	56.6	3.5×10	6.7×10	$5.2\times10 \pm 0.4\times10^{a}$
Burger	13	43.3	1.0×10	8.0×10	$3.2\times10 \pm 0.8\times10^{b}$
Shawerma	11	36.6	1.4×10	5.6×10	$2.6\times10 \pm 0.4\times10^{b}$
Luncheon	20	66.6	1.2×10	3.3×10	$1.9\times10 \pm 0.3\times10^{b}$
Total	61	50.8**			

Values within a column with different superscript letters were significantly different at ($P \leq 0.05$). * Percentage in relation to total number of each sample (30). ** Percentage in relation to total number of samples (120).

From the point of confirmatory molecular detection of enterotoxigenic S. aureus isolates identification, photo (1) proved that out of eight examined isolates, one isolate carried SeA gene, other carried SeB gene, and one carried both SeA and SeD genes were detected.

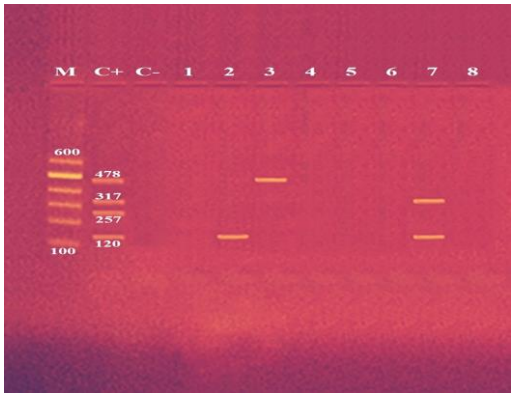

Fig. 1 Agarose gel electrophoresis of multiplex PCR of sea (120 bp), seb (478 bp), sec (257 bp) and sed (317 bp) enterotoxin genes for characterization of S. aureus. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for sea, seb, sec and sed genes. Lane C-: Control negative.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

4. DISCUSSION

Ready to eat fast food meat meals are highly demanded and consumed, but it may be exposed to many sources of contamination beginning from high bacterial loaded raw materials, undercooking, unhygienic practices in handling, hot holding and serving; Staphylococci are one of the most prevalent bacterial contaminants which was confirmed by USFDA (2004) who reported that S. aureus is ubiquitous in nature and inhabits in the mucous membranes and skin of most warm-blooded animals, including food animals and humans; up to 50% of humans may carry this organism in their nasal passages and throats and on their hair and skin.

Staphylococcus aureus as an indicator of contamination of processed foods could come from the skin of handlers (Acco et al., 2003). In developing countries, street vending of foods is common because it offers inexpensive fast foods at convenient locations; in contrast to their potential benefits, concerns over the safety and quality of these foods have been raised, because the vendors lack appreciation of basic food safety issues (Bryan et al., 1988; Ekanem, 1998; Umoh and Odaba, 1999); a number of data confirm the fact that S. aureus causes many outbreaks of food poisoning resulting from hand contact (Bryant et al., 1988).

The incidence and count results of total staphylococci in examined samples as mentioned in table (1) revealed that staphylococci were detected in 67.5% of all examined samples, represented by 73.3, 63.3, 50.0, and 83.3% with mean values $3.42x10^3$, $2.81x10^3$, $1.53x10^3$, and $0.20x10^3$ CFU\g for kofta, burger, shawerma, and luncheon, respectively, which were in somewhat agreement with those reported by Rawash (2015) who recorded that mean values of total staphylococcal count in examined kofta and burger samples were 2.31×10^3 and1.57×10^3 CFU\g, respectively; Abd Allah-Mona (2016) who recorded that the mean value of total staphyloccal count in examined shawerma and kofta samples were $3.9x10^3$, $6.4x10^3$CFU\g, and Elshebacy (2017) who recorded that the mean value of total staphylococcal count in luncheon samples was $2.04x10^3$ CFU\g; while they were different with those recorded by Abd Allah-Enas (2011) who found the mean value of total staphylococcal count in examined kofta sample was $9.14x10^2$ CFU\g; Khater et al. (2013) who recorded the mean value of total staphylococcal count in kofta samples $1.5x10^4$ CFU\g; Youness (2018) ($2.07x10^2$ CFU\g for luncheon samples), and Bahbah (2019) ($1.87x10^2$ CFU\g for burger samples).

In addition, the incidence and\or counts of S. aureus in examined as mentioned in table (2)revealed detection of S. aureus in 50.8% of all examined samples, where kofta was also the most contaminated samples with S. aureus followed by burger, shawerma, and luncheon with mean values 5.2x10, 3.2x10, 2.6x10 and 1.9x10 CFU\g, with incidences of 56.6, 43.3, 36.6, and 66.6%, respectively, which were somewhat agreed with those reported by Rawash (2015), Laban (2018) who recorded that the incidence of S. aureus in their examined RTE kofta, burger, shawerma, and luncheon was 60, 46.6, 40, and 60%, respectively; while they were different with that recorded by Ali and Abd-Elaziz (2011) who recorded that the average of S. aureus count in examined kofta, and shawerma samples were $7.2x10^4$ and $8.98x10^3$ CFU\g, respectively; Shaltout et al. (2015), who recorded that the average of S. aureus count in examined burger samples was $7.54x10^2$ CFU\g, respectively and Morshdy et al. (2018) who found the mean values of S. aureus in examined kofta, luncheon, burger, and shawerma samples were $2.04x10^3$, $7.2x10^3$, $1.9x10^3$, and $8.3x10^3$ CFU/g, respectively.

The variation in the results between different authors may be due to the differences in hygienic measures during manufacturing practices, handling, and the effectiveness of thermal treatment applied during preparation. The presence of such pathogenic bacterium in RTE foods is considered as an indicator of faults during preparation, handling, storage or service which may come through using of

contaminated raw materials, food handlers and the surrounding environment; moreover, it is obvious that kofta samples were the most contaminated beef product which may be referred to under cooking or insufficient heat treatment, and over unhygienic handling post cooking.

Staphylococcus aureus enterotoxins are the major virulence factor causing food poisoning by ingestion of foods contaminated with S. aureus heat-stable enterotoxins; the main SEs incriminated in SFP are staphylococcal enterotoxin A (SeA), staphylococcal enterotoxin B (SeB), staphylococcal enterotoxins C (SeC), and staphylococcal enterotoxins D (SeD); Staphylococcus aureus enterotoxin type A is the most common cause of SFP worldwide, but the involvement of other classical SEs (SeB to SeE) have been also recorded which made PCR detection of enterotoxigenic S. aureus is essential to identify staphylococcal food poisoning (Chiang et al., 2008; Argudin et al., 2010).

Results of molecular detection of S. aureus isolates carrying enterotoxin genes proved that SeAgene was the most prevalent detected; in addition, out of eight examined isolates, one isolate carried SeA gene, other carried SeB gene, and one carried both SeA and SeD genes were detected; results were compatible with Ali and Abd-Elaziz (2011), Naguib (2017), Rezk (2017), Morshdy et al. (2018)who recorded detection of entero-toxigenic S. aureus isolates carrying different enterotoxin genes from ready to eat meat products using multiplex PCR.

5. CONCLUSIONS

The high prevalence of *S. aureus* among the tested samples, mainly in kofta samples, and the presence of the toxigenic *S. aureus* in prepared foods highlighted the necessity of enforcing hygienic practices within fast food and street vended foods kitchens. In the future, the molecular and ecological characterization of isolated toxigenic *S. aureus* strains must be performed to determine the origin of the contamination. Better knowledge of strict hygienic practices during the collection of raw materials, preparation of food, holding, storage, and serving must be educated to food handlers.

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