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Incidence of Staphylococcus enterotoxins in some meals served in Egyptian hotel

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ABSTRACT

A total 120 random samples of beef kofta, chicken panne and fish based meal (sushi) (40 of each) , served in Egyptian hotels located in Cairo Governorate, Egypt were collected to determine of *S. aureus* counts, isolation , identifications and detection of their enterotoxins in the examined samples. The obtained results revealed that *S. epidermidis*, *S. intermedius*, *S. saprophyticus* and *S. xylosus*were recorded in (25%), (2.5%), (0%) and (12.5%) of Kofta , while for chicken panne were detected (37.5%), (10 %), (7.5%) and (2.5 %) , in add to (45%), (17.5%), (5%) and (10 %) were detected in Sushi fish samples, furthermore *S. aureus* was detected in 13(32.5%), 21(52.5%) and 27(67.5%) of the examined kofta, panne and sushi samples. Regarding to the edibility of the examined ready to eat (RTE) samples in relation to its content of *S. aureus*, 11(27.5%), 21(52.5%) and 27(67.5%) of the examined kofta, panne and sushi samples, respectively, were rejected for exceeding *S. aureus* permissible limit (not more than 10^2 cfu/gm). The results also recorded the main values of *S. aureus* counts in the examined kofta, panne and sushi samples were $9.41\times10^2\pm 2.12\times10^2$, $3.27\times10^3\pm 0.54\times10^3$and $5.86\times10^3\pm 0.97\times10^3$cfu/g, respectively. Moreover the incidence for *S. aureus* enterotoxins positive samples, revealed that SE-A was 1(2.5%), 2(5%) and 4(10%) of the examined kofta, panée and sushi samples, respectively; while SE-B was detected in 1(2.5%) of chicken panée only, while not detected in kofta and sushi samples, SE-D was detected in 1(2.5%) each of kofta and sushi sample , while not detected in panne, SE-A+C was detected in 1(2.5%) of kofta samples while not detected in panne and sushi samples, SE-A+D were detected in 1 (2.5%) each of panne and sushi samples while not detected in kofta samples. Overall, 3(7.5%), 4(10%) and 6(15%) of the examined kofta, panne and sushi. Wherever to the highly obtained results of the *S. aureus* prevalence, especially enterotoxigenic strains; it encourages investigation of the antibiotic resistance profile molecularly against erythromycin (*ermA*), gentamicin (*aac 6-aph 2*), methicillin (*mecA*) and vancomycin (*vanA*) resistance genes in randomly collected five *S. aureus* isolates of each sample and results revealed that out of the examined 15 isolates, 8(53.3%), 5(33.3%), 4(26.6%) and 2(13.3%) were harbored *ermA, aac 6-aph 2, mecA* and *vanA* resistance genes, where sushi isolates had the highest prevalence of antibiotic resistance followed by panée and kofta samples, respectively.

1. INTRODUCTION

Pathogenic microorganisms are widely present in soil, water, animals and people. It presents on hands, cloths, utensils and cutting boards, the slight contact can transfer them to meat and cause food-borne diseases. Moreover, Raw food, especially meat, poultry and their extracts have dangerous microorganisms can be transferred into other food during preparation, handling and storage (FAO/WHO,2003 and Hanson *et al.*, 2011).

Meat meals can be exposed to several ways of contamination through improper practices during production, storage and handling. This risk of contamination to these meals increasing by storage of food at ambient temperature, by using insufficiently high temperature for re-heating the food and also adding contaminated ingredients at stage which no further heat treatment applied (Ehirl *et al*., 2001).

The risk of bacterial food-borne disease also increases when meat meals were prepared in kitchens of student accommodation, youth hostels and shared homes. This increase of the risk may be due to high number of individuals who work in the kitchens without take care the safety issues and follow hygienic regulation.

Poisoning bacteria grow in food rapidly in the danger zone, so handlers are advised to never leave food out of refrigeration more than two hours. in case of the temperature is above 32°C, food must not be left out more than one hour (FSIS, 2008).

S. aureus consider the main source of bacterial contamination in cooked meat due to workers handling during preparation and processing of it (FSIS, 2013). In addition, *S. aureus* were detected in 359 outbreaks and sporadic cases in the United Kingdom for production of

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enterotoxin and recorded that meat and meat-based dishes was the vehicle of 75% of the incidents (FAO/WHO,2003).
Antibiotic resistance is a global public health threat emerged vigorously in the last decades which attributed mainly to wiseless administration of antibiotics. Staphylococcus aureus is one of main reported multi-drugs resistant bacteria (MDR) that has been attributed to the presence of some antibiotic resistant genes, especially *mecA* gene which encoded penicillin-binding protein (Tambekar *et al.*, 2011).
PCR technology is the most promising due to rapidity, economical and sensitivity, since it can detect many micro-organisms in clinical samples. Recently, specific oligonucleotide primers for PCR have been described for analysis of multi-drugs resistant *S. aureus* strains (Beck*et al.,* 2006).
Therefore, the current study was planned out to evaluate *S. aureus* count and detection the enterotoxins of some examined meat meals in Egyptian hotels through the following:

1- Determination of Staphylococci counts.
2- Detection and types of Staphylococcus species.
3- Detection and typing of enterotoxin.
4-Molecular detection of some antibiotic resistant genes in some isolated *S. aureus* strains.

2. MATERIAL AND METHODS

Preparation and collection of samples:
A total of 120 random meat meal samples represented by beef kofta, chicken panne, and fish-based meal (sushi) (40 of each) served in the Egyptian hotels located in Cairo governorate, Egypt was collected. Because the Egyptian hotels serve hundreds of RTE (thermally treated or not) food items, the selected samples were obtained to investigate *S. aureu* and their toxins and assess the health risk of them.
Preparation (ISO 6887-1, 2017):
Prepare 25 grams of examined sample, then add 225 ml of sterile peptone water were added and thoroughly mixed using homogenizer for 2 minutes, from which ten-fold serial dilutions prepared. The prepared samples were subjected to the following examinations:
Determination of *S. aureus* counts (ISO 6888-1:1999, A1:2003):
0.1 ml from each of the previously prepared dilutions was spread over Baird Parker agar plate. The inoculated and control plates were incubated at 37°C / 48 hours. black, shiny, circular, smooth, convex colonies with narrow white margin and surrounded by a clear zone extending into opaque medium were enumerated (suspected *S. aureus*) and total count/g was calculated. Then these colonies were picked up and purified on Semi-solid agar slopes for morphological examination and biochemical identification.
Identification of detected strains:
1. Morphological identification (ISO 6888-1:1999, A1:2003).
2. Biochemical test for identification (MacFaddin, (2000):
3. Detection and typing of enterotoxin (Shingaki et al., 1981):
4. Polymerase Chain Reaction (PCR) (Perez et al., 2001):

3. RESULTS

Table 1 Incidences of Staphylococcus species isolated from examined samples (n=40)

Beef Kofta	Beef Kofta		Chicken Panne		Sushi Fish	
Strains	No.	%	No.	%	No.	%
S. aureus	13	32.5	21	52.5	27	67.5
S. epidermidis	10	25	15	37.5	18	45
S. intermedius	1	2.5	4	10	7	17.5
S. saprophyticus	0	0	3	7.5	2	5
S. xylosus	5	12.5	1	2.5	4	10

Table 2 Staphylococcus aureus count/g in the examined meat meal samples (n=40)

Meal	Min	Max	Mean ± S.E*
Beef Kofta	1.0×10^2	4.0×10^3	$9.41\times10^2\pm 2.12\times10^2$
Chicken Panne	2.0×10^2	9.0×10^3	$3.27\times10^3\pm 0.54\times10^3$
Sushi Fish	5.0×10^2	3.0×10^4	$5.86\times10^3\pm 0.97\times10^3$

Table 3 Edibility of the examined samples of meals served in the Egyptian hotels based on their contamination with *S. aureus* (n=40).

Accepted samples Meals	Accepted samples No.	%	Unaccepted samples No.	%	*Staph. aureus*/g*
Beef Kofta	29	72.5	11	27.5	$\geq 10^2$
Chicken Panne	19	47.5	21	52.5	$\geq 10^2$
Sushi Fish	13	32.5	27	67.5	$\geq 10^2$
Total	61	51%	59	49 %	

*Egyptian Organization for Standardization (2005)

Table 4 Incidences of *S. aureus* enterotoxin isolated from meals served in the Egyptian hotels

Enterotoxin	Beef Kofta		Chicken Panne		Sushi Fish	
	No	%	No	%	No	%
A	1	2.5	2	5	4	10
B	-	-	1	2.5	-	-
D	1	2.5	-	-	1	2.5
A+C	1	2.5	-	-	-	-
A+D	-	-	1	2.5	1	2.5
Total	3	7.5%	4	10%	6	15%

Table 5 Incidence of antibiotic resistant genes of *S. aureus* using multiplex PCR (15 strains)

Antibiotic resistant genes	No of strains		*vanA*		*mecA*		*aac (6)-aph*		*ermA*	
		%	No.	%	No.	%	No.	%	No.	
Beef Kofta	5	0	0	2.5	1	0	0	2.5	1	
Chicken Panne	5	2.5	1	0	0	2.5	1	7.5	3	
Sushi Fish	5	2.5	1	7.5	3	10	4	10	4	
Total	15	5 %	2	10%	4	12.5%	5	20%	8	

4. DISCUSSION

Staphylococcus aureus are carried on human hands, nasal passages and throats. Most food borne illness outbreaks are a result of contamination from food handlers and production of heat stable toxins in the food, bad sanitary food handling and improper cooking sources of Staphylococcal illnesses (FSIS 2003).

Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination, by manual contact or through respiratory secretions. *S. aureus* is a common present in the skin and mucosal membranes of humans, (Kluytmans *et al.*, 2005).

Staphylococcus species had been investigated in the examined samples. The results recorded intable (1) revealed that detection of *S. aureus*, *S. epidermidis*, *S. intermedius*, *S. saprophyticus* and *S. xylosus*in 61(50.83%), 43(35.83%), 12(10%), 5(4.16%) and 10(8.33%) of the examined samples; where pathogenic *S. aureus* was detected in 13(32.5%), 21(52.5%) and 27(67.5%) of the examined kofta, panne and sushi samples with mean counts of $9.41\times10^2\pm 2.12\times10^2$, $3.27\times10^3\pm 0.54\times10^3$and $5.86\times10^3\pm 0.97\times10^3$CFU/g, respectively (Table 2).

Regarding to the previously recorded results, Hassan *et al.* (2015) (26.67% of the examined kofta samples), Hassanin *et al.* (2015) (60% of the examined RTE kofta samples), Liang *et al.* (2016) who detected *S. aureus* in 34.2% of the examined sushi samples with mean count of $1.9x10^2$ CFU/g), Shaltout *et al.* (2018) (56.6% of the examined panne samples), Saif (2019) (5.2x10 CFU/g with prevalence of 56.6% of the examined kofta samples), and Gaafar (2020) ($12x10^3$ and $9.2x10^3$ CFU/g with prevalence of 40 and 36.6% of kofta and panne samples, respectively). On the other hand, Hoel *et al.* (2015) (did not detect *S. aureus* in the examined sushi samples).

Referring to the edibility of the examined RTE samples in relation to its content of *S. aureus*, 11(27.5%), 21(52.5%) and 27(67.5%) of the examined kofta, panne and sushi samples were rejected for exceeding *S. aureus* permissible limit (not more than 10^2 CFU/g) (Table, 3).

Variation between authors may be referred to differences in personal hygiene, storage, temperature and handling conditions, staphylococcal food poisoning is the result of performed heat stable enterotoxins that are produced by certain strains of *S. aureus* resulting in symptoms of food intoxication. The main enterotoxins involved in SFP are *sea* (Staphylococcal enterotoxins A), *seb* (Staphylococcal enterotoxins B), *sec* (Staphylococcal enterotoxins C), *sed* (Staphylococcal enterotoxins D) and *see* (Staphylococcal enterotoxins E) (Chiang *et al.*, 2008).

Table (4) showed the incidence of SEs detection in positive samples; the results revealed detection of SE-A in 1(2.5%), 2(5%) and 4(10%) of the examined kofta, panne and sushi samples, respectively; while SE-B was detected in 1(2.5%) of chicken panne only while not detected in kofta and sushi samples, SE-D was detected in 1(2.5%) of kofta and sushi samples of each while not detected in panne samples, SE-A+C was detected in 1(2.5%) of kofta samples while not detected in panne and sushi samples, SE-A+D were detected in 1 (2.5%) each of panne and sushi samples while not detected in kofta samples. Overall, 3(7.5%), 4(10%) and 6(15%) of the examined kofta, panne and sushi contained SEs; in addition, SE-A was the prominently detected SE which proved that SE-A is the most common staphylococcal enterotoxin detected in food samples as reported by Argudín *et al.* (2010).

Previous investigations conducted by Kim *et al.* (2011), Saif (2019), and Gaafar (2020) could detect SEs in RTE sushi, kofta and panne samples, respectively, where SEA was the most prominent detected enterotoxin.

Referring to the highly obtained results of the *S. aureus* prevalence, especially enterotoxigenic strains; it encourages investigation of the antibiotic resistance profile molecularly against erythromycin (*ermA*), gentamicin (*aac 6-aph 2*), methicillin (*mecA*) and vancomycin (*vanA*) resistance genes in randomly collected five *S. aureus* isolates of each sample. Results revealed that out of the examined 15 isolates, 8(53.3%), 5(33.3%), 4(26.6%) and 2(13.3%) isolates harbored *ermA*, *aac 6-aph 2*, *mecA* and *vanA* resistance genes, where sushi isolates had the highest prevalence of antibiotic resistance followed by panne and kofta samples, respectively (Table, 5).

5. CONCLUSION

For the differences between different authors in the field of the bacteriological quality of the examined RTE samples may be referred to variation in the localities, time and conditions of collection, also, it may refer to variation in the personal hygiene, processing and storage conditions, and hygienic quality of the manufacturing facilities.

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