



Staphylococci in some meat products

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ABSTRACT

Meat products are liable to harbor different types of micro-organisms during long chain of handling, processing, distribution and storage as well as preparation, so a total of 90 random samples of locally manufactured meat products represented by frozen beef burger (30), kofta (30) and sausage (30) "25 grams of each" were randomly collected from different shops in El-Menoufia governorates, Egypt. It is evident from the obtained results that all the examined samples of beef burger, kofta and sausage were positive Staphylococci. On the other hand, *S.aureus* detected in 40%, 46.67% and 63.33% in examined samples of beef burger, kofta and sausage, respectively. Serological examination declared that the incidence of Staphylococcus species from examined beef burgers were 40%, 23.33% and 3.33% for *S.aureus*, *S.epidermidis* and *S.intermedius*, respectively. While, in kofta were 46.67%, 26.67% and 6.67% for *S.aureus*, *S.epidermidis* and *S.saprophyticus*, respectively and in sausage were 63.33%, 36.67%, 10%, 13.33 and 6.67 for *S.aureus*, *S.epidermidis*, *S.intermedius*, *S.saprophyticus* and *S.xylosum*, respectively. Whilst, the detected *S.aureus* enterotoxins from examined samples were enterotoxin B in beef burger with incidence of 3.33%, enterotoxin A and enterotoxin C with incidence of 3.33% and 3.33, respectively from kofta samples. While in sausage sample enterotoxin A, B and D&C were detected with incidence of 10%, 3.33% and 3.33%, respectively. The presence of these microorganisms in large numbers make these meat products of inferior quality and unfit for human consumption.

Key words: Staphylococci, *S. aureus*, beef burger, kofta, sausage, Enterotoxins.

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1. INTRODUCTION

Foods of animal origin including meat are required to maintain the health of human body (Nestle, 1999) and they are highly demanded due to their high biological value, responsible price, agreeable taste and easily serving. On the other hand these benefits come over the safety and the quality of such food items because the vendors have lack of information about the basic food safety rules (Gibbons *et al.*, 2006).

Foodborne infection and intoxication outbreaks are increasing day by day in industrial and developing countries, the majority of cases of foodborne diseases were

due to bacterial agents (Stevenson and Bernard, 1995). Staphylococcus spp can be carried on hands, nasal passage or throats. Most food borne illness outbreaks are result of contamination from food handlers and production of heat stable toxins in food. (FSIS, 2003) and the most common way for food contamination with Staphylococcus is through contact with food workers who carry the bacteria. (CDC, 2006).

Staphylococcal food poisoning is the result of preformed enterotoxins that are produced by certain strains of *S.aureus* resulting in symptoms of intoxication, not

infection. The most common symptoms appear approximately 3-8 hrs after ingestion and include nausea, vomiting, abdominal cramps and diarrhea. Generally, symptoms are short in duration (approximately 24 - 48 hrs) (Sandel and Mckillip, 2004).

Therefore, this work was planned out to study the bacteriological contamination in some locally manufactured meat products from different local commercial shops in Menoufia government, Egypt to investigate the following: Determination of Staphylococci and *S. aureus* count; and isolation and then identification of Staphylococcal species, *S.aureus* and some of its enterotoxins.

2.MATERIAL AND METHODS

2.1. Collection of samples:

Ninety random samples of locally manufactured meat products represented by frozen beef burger(30), kofta(30) and sausage(30) of 25 grams from each examined sample were collected from different shops supermarkets in Menoufia government, Egypt. All collected samples were separately kept in sterile plastic bag and transferred in an ice box to the laboratory under complete aseptic conditions without undue delay. The samples were subjected to the bacteriological examination for detection of *Staphylococcus aureus* in such products.

2.2.Preparation of samples (ICMSF, 1996).

2.3. Determination of Staphylococci and *S. aureus* count (FDA, 2001).

The method described by (FDA, 2001) and the developed colonies appeared on Baird Parker agar plate after incubation at 37°C for 48 hours. The suspected colonies of *Staph .aureus* appear as black, shiny, circular, smooth, convex with narrow white margin and surrounded by a clear zone extending into opaque medium were enumerated and *Staphylococcus aureus* count /gwas calculated. Also, the colonies

were picked up and purified on nutrient agar slopes for further morphological, biochemical and serological examination identification.

2.4. Identification of Staphylococci species.

2.5. Detection of *Staphylococcus aureus* enterotoxins.

The serologically identified *S. aureus* strains were examined for their ability to produce enterotoxins using Staphylococcal Enterotoxin –Reverse Passive Latex Agglutination kit (SET-RPLA) and Sac culture method recommended by (Oda et al., 1979).

2.6. Detection and typing of *S. aureus* enterotoxin (Shingaki, et al., 1981):

The clear culture supernatant fluid was tested serologically by RPLA technique using kits for the detection of staphylococcal enterotoxins A, B, C and D (SET-RPLA, Denka Sekeu LTD, Japan for OxoidLTd).

2.7. Statistical analysis: according to Feldman et al. (2003).

3. RESULTS

It is evident from the results recorded in table (1) that all the examined samples were positive staphylococci with mean value (cfu/g) $4.07 \times 10^3 \pm 0.69 \times 10^3$ for beef burger, $9.52 \times 10^3 \pm 2.14 \times 10^3$ for kofta and $2.38 \times 10^4 \pm 0.51 \times 10^4$ for sausage, respectively and according to Egyptian Organization for Standardization " EOS " ,the total Staphylococci count /g should be not more than 10^2 (cfu/g), therefore 64%, 88% and 44% of examined beef burger, kofta and sausage respectively, were unaccepted as recorded in table (2).

On the other hand, *S.aureus* detected in 12(40%), 14(46.67%) and 19(63.33%) in examined samples of beef burger, kofta and sausage, respectively with mean value of $8.71 \times 10^2 \pm 1.49 \times 10^2$ (cfu/g) for beef burger, $3.10 \times 10^3 \pm 0.74 \times 10^3$ (cfu/g) for kofta and $5.96 \times 10^3 \pm 0.88 \times 10^3$ (cfu/g) for sausage, respectively as recorded in (table 3).

Seriological examination declared that the incidence of Staphylococcus species from examined samples of locally manufactured meat products is 40%, 23.33% and 3.33% for *S.aureus*, *S.epidermidis* and *S.intermedius*, respectively could be isolated from beef burger, 46.67%, 26.67% and 6.67% for *S.aureus*, *S.epidermidis* and *S.saprophyticus*, respectively could be isolated from kofta and 63.33%, 36.67%, 10%, 13.33% and 6.67% for *S.aureus*, *S.epidermidis*, *S.intermedius*, *S.saprophyticus* and *S.xylosum*, respectively could be isolated from sausage samples as recorded in (table 4) and the isolation percent

was calculated according to the number of examined samples .

While, the incidence of *S.aureus* enterotoxins isolated from examined samples of locally manufactured meat products were enterotoxin B from beef burger with incidence of 3.33%, enterotoxin A (one sample) and enterotoxin C (one sample) with incidence of 3.33% and 33.3%, respectively from kofta samples, while enterotoxin A(3 samples), B(one sample) and D&C (one sample) were isolated from sausage samples with incidence of 10%, 3.33% and 3.33% respectively as recorded in (table 5).

Table (1): Incidence and Staphylococci count/g in the examined samples of locally manufactured meat products (n=30).

Meat Products	+ve samples		Min	Max	Mean \pm S.E*
	No.	%			
Beef burger	30	100	1.0×10^2	8.6×10^3	$4.07 \times 10^3 \pm 0.69 \times 10^3$
Kofta	30	100	2.0×10^2	1.9×10^4	$9.52 \times 10^3 \pm 2.14 \times 10^3$
Sausage	30	100	5.0×10^2	7.2×10^4	$2.38 \times 10^4 \pm 0.51 \times 10^4$

S.E* = Standard error of mean

Table (2): Acceptability of the examined samples of locally manufactured meat products based on their Staphylococci counts/g (n=30).

Products	Staphylococci count /g	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Beef burger	$>10^2$	9	36	16	64
Kofta	$>10^2$	3	12	22	88
Sausage	$>10^2$	14	56	11	44

Egyptian Organization for Standardization "EOS" (2005).

No 1688-2005 for beef burger

No 1973-2005 for kofta

No 1972-2005 for sausage

Table (3): Incidence of *S. aureus* count/g in the examined samples of locally manufactured meat products (n=30).

Meat Products	+ve samples		Min	Max	Mean \pm S.E*
	No.	%			
Beef burger	12	40	1.0×10^2	2.1×10^3	$8.71 \times 10^2 \pm 1.49 \times 10^2$
Kofta	14	46.67	1.0×10^2	6.5×10^3	$3.10 \times 10^3 \pm 0.74 \times 10^3$
Sausage	19	63.33	1.0×10^2	1.3×10^4	$5.96 \times 10^3 \pm 0.88 \times 10^3$

S.E* = Standard error of mean

Table (4): Incidence of Staphylococcus species isolated from the examined of samples locally manufactured meat products (n=30).

Meat products Staphylococcus Species	Beef burger		Kofta		Sausage		Total (n=90)	
	No.	%	No.	%	No.	%	No.	%
<i>Staphylococcus aureus</i>	12	40	14	46.67	19	63.33	45	50
<i>Staphylococcus epidermidis</i>	7	23.33	8	26.67	11	36.67	26	28.89
<i>Staphylococcus intermedius</i>	1	3.33	0	0	3	10	2	6.67
<i>Staphylococcus saprophyticus</i>	0	0	2	6.67	4	13.33	5	16.67
<i>Staphylococcus xylosum</i>	0	0	0	0	2	6.67	1	3.33

N.B.

The isolation % was calculated according to the number of examined samples

Table (5): Incidence of enterotoxins of *S. aureus* isolated from the examined samples of locally manufactured meat products.

Enterotoxin	Beef burger (30)		Kofta (30)		Sausage (30)		Total (90)	
	No.	%	No.	%	No.	%	No.	%
A	0	0	1	3.33	3	10	4	4.44
B	1	3.33	0	0	1	3.33	2	2.22
C	0	0	1	3.33	0	0	1	1.11
D & C	0	0	0	0	1	3.33	1	1.11
Total	1	3.33	2	6.67	5	16.67	8	8.89

4. DISCUSSION:

Meat products are gaining popularity because they represent quick easily prepared meals of low price from one side and render the processors to convert the various types of meat into unified products. On the other side, meat products are liable to harbour different types of microorganisms a long the chain through handling, processing, distribution and storage as well as preparation (Hassanien, 2004). Further foods from animal origins are naturally susceptible to contamination by *S.aureus* which then multiply and produce enterotoxins (Morales *et al.*, 2009).

According to table (1), nearly similar results were reported by (Abd El-Hamid, 2010) with incidence of $2.2 \times 10^3 \pm 4.54 \times 10^2$ and $2.17 \times 10^3 \pm 4.31 \times 10^2$ cfu/g, for sausage and beef burger, respectively and the higher results were reported by (Abou Hussein, 2004) $5.38 \times 10^5 \pm 9.7 \times 10^4$ and $9.6 \times 10^5 \pm 2.1 \times 10^5$ for sausage and beef burger, respectively, (Talaat, 2009) $1.04 \times 10^7 \pm 4.3 \times 10^6$ (cfu/g) and $1.46 \times 10^6 \pm 5.33 \times 10^5$ (cfu/g) for sausage and beef burger, respectively and (Ibrahim, 2015) $1.97 \times 10^5 \pm 6.49 \times 10^4$ and $2.08 \times 10^5 \pm 5.56 \times 10^4$ (cfu/g) for examined sausage and beef burger, respectively. While lower result recorded by (Abd El Satter, 2016) with incidence of $3.05 \times 10^3 \pm 0.97 \times 10^3$ (cfu/g) for kofta samples.

Concerning the results of table (3) nearly similar results for beef burger samples were recorded by (Ibrahim, 2016) 30% positive samples with incidence of $1.02 \times 10^4 \pm 2.53 \times 10^3$ cfu/g, (Hassanien, 2004) 36% positive samples with incidence of $6.34 \times 10^3 \pm 1.02 \times 10^3$ /g (Radwan, 2009) 36% and (Ahmed, 2011) 36% for the same examined product. While, lower results were recorded by (Ouf, 2001) 20%, (Essa and Makar, 2003) 18%, (El said, 2005) 4%, (Salah and Salah Eldeen, 2005) 10%, (Shahrazet *et al.*, 2012) 25%, (Eldaly *et al.*, 2014) 10% and

(Atiah, 2017) 15% and higher results were recorded by (Al-kour, 2001) $2.27 \times 10^3 \pm 0.82 \times 10^3$ cfu/g and (Abd El-Hady, 2015) recorded 50% positive *S. aureus* samples in the same product. On the other hand, concerning to kofta samples lower results were recorded by (Hassanien, 2004) 24% positive samples for the suspected *S.aureus* with mean value of $2.51 \times 10^3 \pm 0.31 \times 10^3$ (cfu/g) and (Abd El Satter, 2016) with mean value of $4.3 \times 10^2 \pm 2.06 \times 10^2$ (cfu/g). For the sausage samples: nearly similar results recorded by (Hassanien, 2004) 52% positive samples with mean value of $1.12 \times 10^3 \pm 0.17 \times 10^3$ (cfu/g) while, lower results recorded by (Ibrahim, 2016) (22%) positive *S.aureus* samples with mean value of $3.03 \times 10^3 \pm 6.33 \times 10^2$, (Eldaly *et al.*, 2014) 20% positive sausage samples with mean value of $2.1 \times 10^4 \pm 0.5 \times 10^4$, (Ouf, 2001) 10%, (Farid, 2001) 20%, (El said, 2005) 10%, (Salah and Salah Eldeen, 2005) 16.6%, (Esteves *et al.*, 2006) 40%, (Mohamed, 2009) 31.43%, (Seham *et al.*, 2013) 20% and (Armany, 2016) 24% and higher result recorded by (Abou Hussien, 2004) 72% positive *S.aureus* in examined sausage samples.

Furthermore, *S.aureus* was isolated previously by (Ibrahim, 2016); (Armany, 2016) and (Abou Hussien, 2004) from sausage samples, (Abd El Satter, 2016) and (Hassanien, 2004) from beef burger and kofta samples, while *S.epidermidis*, *S.intermedius*, *S.saprophyticus* were isolated from beef burger and kofta samples by (Abd El Satter, 2016) with incidence rate of 42%, 16% and 26% in beef burger & 22%, 34% and 12% in kofta samples, respectively.

Staph. aureus may produce numerous numbers 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) and considered as 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 meat products (Güven *et al.*, 2010).)and the symptoms of staphylococcal food poisoning are abdominal cramps, nausea, vomiting, sometimes followed by diarrhea (never diarrhea alone). The onset of symptoms remission is observed after 24h (Le Loir, 2003).

Presence of *S.aureus* in meat products may be attributed to direct contact with workers with hand or arm lesions caused by *S.aureus*, or by coughing and sneezing which is common during respiratory infections. Food handlers are frequently the source of food contamination in staphylococcal outbreaks (Jennifer Hait, 2012)so, microorganisms control in meat products is the major concern in the preparation of high quality foods especially during slaughtering process as the meat is exposed to many sources of contamination such as hands of workers, water, air and equipments (Jo *et al.*,2004) and sanitation programs address issues such as personal hygiene, hygienic work practices, employee education and proper cleaning and sanitization protocols. Knives, gloves and aprons are among a number of pieces identified as potential reservoirs of bacteria if not cleaned or sanitized properly (Dean, 2007).

5. CONCLUSION

This study concluded that the examined meat products of (beef burger, kofta and sausage) from local commercial retail shops in Menofia government harbor a high microbial loads of *S.aureus* and this is due to contamination during different processing stages or due to using of contaminated materials as spices or contamination during handling and from surrounding and poor sanitary conditions which lead to inferior quality product and become unfit for

consumption. Also *S. aureus* enterotoxins which were detected in the examined samples have public health hazard.

S. aureus currently attracted increasing attention due to its capability of producing a range of enterotoxins and tissue degrading enzymes and become a leading cause of food poisoning resulting from the consumption of contaminated food with staphylococcal enterotoxins, therefore, microorganisms control in meat products is the major concern in the preparation of high quality foods.

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