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OCCURRENCE OF *STAPHYLOCOCCUS AUREUS* IN FAST FOOD WITH SPECIAL REFERENCE TO ITS ENTEROTOXIGENICITY

(With 4 Tables)

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تواجد الميكروب المكور العنقودى الذهبى فى الوجبات السريعة مع أشارة خاصة لسميتة المعوية

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أجريت هذه الدراسة لمعرفة مدى تلوث الوجبات السريعة (ساندويتشات) المجهزة للأكل من منتجات اللحوم (شاورمة – سجق – برجر) والمجمعة من محلات وبائعى الوجبات السريعة بمدينة بورسعيد بميكروب المكور العنقودى الذهبى وكذلك للوقوف على معرفة مدى إفراز العترات المعزولة للسموم المعوية المسببة للتسمم الغذائى. ولقد شملت الدراسة إجراء الفحص البكتيريولوجى لعدد 75 عينة حيث أفضت الى وجود الميكروب المكور العنقودى الذهبى بنسب 32%، 44%، 36% وكذا كان العد البكتيرى للميكروب 9.8×10^2 ، 1.2×10^3 ، 8.3×10^2 فى كل من الشاورمة والسجق والبرجر على التوالى. وتم تصنيف العترات المعزولة لمدى إفرازها للسموم ولوحظ أن نوع السم A , D الأكثر وجوداً فى العينات المجمعة . ولقد نوقشت الأهمية الصحية للميكروب وسمومه ووضعت التوصيات اللازمة لسلامة المستهلك والمنتج.

SUMMARY

A total of 75 random samples of fast food (ready-to-eat meat sandwiches) represented as 25 each of shawarma, sausage and beef burger were collected from various fast food restaurants and vendors on the street in Port Said city. *S. aureus* could be detected in 32, 44 and 36% of the analyzed samples with a mean value of $9.8 \times 10^2 \pm 0.12 \times 10^2$; $1.2 \times 10^3 \pm 0.24 \times 10^2$ and $8.3 \times 10^2 \pm 0.09 \times 10^2$ CFU/g shawarma, sausage and beef burger, respectively. Out of 8 strains obtained from shawarma, only 3 were enterotoxigenic belonging to type A enterotoxin. Five *S. aureus* were enterotoxigenic obtained from sausage samples, 2 of

them were type A and the other 3 strains were enterotoxins AD. Furthermore, 4 strains identified as 2 type AB producers and 2 type ABCD producers were isolated from beef burger samples. The results showed that enterotoxin A (SEA) was the most frequently in all the examined ready-to-eat-sandwiches followed by enterotoxin type D (SED), indicating that *S. aureus* had a potential public health significance in fast food.

Key words: *Fast food, shawarma, sausage, beef burger, staph aureus*

INTRODUCTION

In Egypt as in many other African countries, fast foods are commonly vended on streets and at market sites in urban area where there is constant and heavy movement of people. Although there are no readily obtainable epidemiological data about the risks of food borne diseases resulting from these foods, the laboratory data show that street vended foods frequently have high microbial populations and occasionally high population of pathogenic bacteria (Bryan, 1988 & 1992).

Nowadays, meat products consumed as sandwiches of shawarma, sausage, beef burger, etc. are commonly prepared and sold by many restaurants which are widely distributed all over the country (Take-away). *Staphylococcus aureus* is one of the most important microorganisms which can contaminate or recontaminate cooked foods via workers hands, equipments or utensils (Bryan, 1978 & 1988). The primary reservoirs of *S. aureus* are human skin and mucosa especially the nasopharyngeal cavity (Bystron *et al.,* 2005).

Staphylococcal food poisoning resulting from the growth of enterotoxigenic strains of *S. aureus* in food leading to the production of enterotoxins which is considered one of the major causes of food borne disease all over the world (Minor and Marth, 1972).

Enterotoxins are groups of single chain protein (poly peptides) with molecular weight 28.000- 35.000 Daltons resistant to high temperature (heat stable) and proteolytic enzymes. The enterotoxigenic strains of *S. aureus* produce several types of enterotoxins (A, B, C, D and E) which can cause symptoms of intoxications such as vomiting, diarhoea and abdominal cramping (Korpysa *et al.,* 2005). Enterotoxin A (SEA) is responsible for a majority of staphylococcal food poisoning whereas enterotoxin B (SEB) is rarely involved (Robbins *et al.,* 1974).

In addition most outbreaks as recorded by Halpin-Dohnalek and Marth (1989) resulted from the combined effect of contamination of the food with *S. aureus* often through unsanitary handling, and holding the food at the wrong temperature thus allowing growth and synthesis of enterotoxins. However, the enterotoxication generally is not lethal and the elderly are more susceptible than younger individuals. The amount of enterotoxin necessary to cause intoxication is very small about 94-184 ng (Erol and Iseri, 2004).

Therefore, the purpose of such investigation is to record the occurrence of *S. aureus* in some different fast meat food and its ability to produce enterotoxins.

MATERIALS and METHODS

1. Collection of samples:

A total of 75 random samples of fast meat food were purchased from different fast food restaurants and vendors on street in Port Said city. The samples were represented as 25 each of ready-to-eat shawarma, sausages and beef burger sandwiches. The samples were directly transferred to the laboratory in an ice box under hygienic conditions without delay to be examined bacteriologically.

2. Preparation of the samples:

According to the technique recommended by APHA (1992), ten grams of each meat product sample only without bread were put into the stomacher bag, to which 90 ml of sterile physiological saline (0.9 %) were aseptically added to provide dilution of 1/10, then the content of the bag was stomached for 60 sec, using the stomacher, then 10 fold serial dilutions were prepared.

3. Determination of *S. aureus* count:

Staphylococcus aureus count/g was determined using surface plating technique according to FAO (1992). 0.1 ml from each of the previously prepared decimal dilutions was transferred onto duplicate plates of Baird- parker media, supplemented with egg yolk tellurite and incubated at 37°C for 24- 48 hrs. Then the black and shiny colonies greater than 1 mm in diameter with narrow white margin surrounded by clear zone extending into opaque medium were counted and recorded.

4. Isolation and Identification of *S. aureus* (FAO, 1992):

Suspected colonies of *S. aureus* were subcultured on slants of brain heart infusion agar (Oxoid) (FAO, 1992) and incubated at 37°C for 24 hrs. Isolated purified strains were identified microscopically and

biochemically for confirmation according to Flandrois and Carret (1981), Marchant and Parker (1983) and Bailley and Scott (1990).

5. Production and detection of enterotoxins from the isolated strains of *S. aureus*:

a- Production of staphylococcal enterotoxins from the isolated strains:

The isolated strains of *S. aureus* were examined for their ability to produce enterotoxins using Sac culture method (Donnelly *et al.*, 1967).

b- Detection and typing of enterotoxins:

According to Oda *et al.* (1979) and Shingaki *et al.* (1981), detection and typing of enterotoxins were done using serological test by reversed passive latex agglutination technique using Oxoid SET- RPLA (kit used for the detection of staphylococcal enterotoxins A, B, C and D).

6. Detection of staphylococcal enterotoxins in the food samples (Direct extraction):

a- Toxin extraction: the steps including preparation of the food samples and toxin extraction from the food itself described by the method recommended by Park and Szabo (1986) were followed.

b- Enterotoxin detection: as mentioned previously methods recommended by Oda *et al.* (1979) and Shingaki *et al.* (1981) was followed using SET- RPLA kit in which antibody- coated latex particles are visibly agglutinated in the presence of SET (Staphylococcal Enterotoxins). It is commercially available in kit form Oxoid Ltd (Basing stoke, Hampshire, UK).

RESULTS

Table 1: Incidence of *S. aureus* in the examined fast food samples.

Fast food samples	No. of examined samples	Positive samples	
		No.	%
Shawarma	25	8	32%
Sausage	25	11	44%
Beef burger	25	9	36%
Chi square			2.00
Probability			0.368*

* Non significant

Table 2: Statistical results of *S. aureus* count /g of the examined fast food samples.

Fast food samples	Min.	Max.	Mean ± S.E.
Shawarma	2×10^2	3.0×10^3	$9.8 \times 10^2 \pm 0.12 \times 10^2$ a
sausage	1×10^2	3.4×10^3	$1.2 \times 10^3 \pm 0.24 \times 10^2$ a
Beef burger	2×10^2	2×10^3	$8.3 \times 10^2 \pm 0.09 \times 10^2$ a
F-calculated			11.357#

Mean of the positive samples.
Significant at $P < 0.05$ using ANOVA test.
a, b unsignificant difference between similar litter at $P < 0.05$ using Duncan Multiple Range Test.

Table 3: Frequency distribution of the examined fast food samples based on their *S. aureus* count.

Intervals	Frequencies in different fast food samples					
	Shawarma		Sausage		Beef burger	
$10\text{-}10^2$	-	-	1	9	-	-
$10^2 - 10^3$	5	62.5	4	36.4	6	66.6
$10^3 – 10^4$	3	37.5	6	54.5	3	33.3
Total	8	100	11	99.9	9	99.9

Table 4: Incidence of enterotoxigenic *S. aureus* and types of its enterotoxins.

Fast food samples	No. of strains tested	Enterotoxigenic strains		Types of enterotoxins						
		No.	%	A	B	C	D	AB	AD	ABCD
Shawarma	8	3	37.5	3	-	-	-	-	-	-
Sausage	11	5	45.5	2	-	-	-	-	3	-
Beef burger	9	4	44.5	-	-	-	-	2	-	2
Chi square			0.884							
Probability			0.643*							

* Non significant

DISCUSSION

The results in Table 1 revealed that *S. aureus* was present in 32, 44 and 36% of shawarma, sausage and beef burger sandwiches, statistically there are no significant difference between the three ready to eat meat products.

It is obvious from such incidence that the level of contamination by *S. aureus* in the examined samples was relatively high, since these fast food are ready-to-eat in the form of sandwiches without any further heat processing. In this respect Wieneke (1974) isolated *S. aureus* (32-36%) from cooked foods including meat and she added that *S. aureus* strains isolated from human resembled those from cooked foods. While, Halpin-Dohnalek and Marth (1989) stated that outbreaks of *S. aureus* food poisoning are most often associated with processed red meats and most outbreaks resulted from the combined effects of contamination of the food with *S. aureus*, often through unsanitary handling and holding the food at the wrong temperature thus allowing growth and synthesis of enterotoxins by the pathogen. Alkanahl and Gasim (1993) found that cooked foods (both unheated and reheated) were associated with more incidents of food borne illness cases (56%) than uncooked foods (20%), *S. aureus* was found in both cooks and suspect foods. It was also found high % of the cooks associated with food borne incidents had no health certificates.

Regarding the incidence of *S. aureus* recorded in Table 1, the obtained results were less than those obtained by El-Daly (1983); El-Sherbeeny *et al.* (1985); Alkanahl and Gasim (1993); Kumar *et al*. (2001) and higher than those obtained by Soriano *et al.* (2000), Little *et al*. (2001); Firinu *et al*. (2003); Shabana and Ouf (2003). Bystron (2005) and Manfreda *et al*., (2005) recorded similar results. On the other hand Hassan (1991), Hegazy (1999) and Ebraheem (2001) can not count *S. aureus* from ready to eat meat sandwiches. Sokari and Anozie (1990) suggested that the high level of contamination with *S. aureus* resulted from cross-contamination reflecting excessive hand contact with food stuffs.

As shown in Table 2 that illustrated the mean values of *S. aureus* count in the examined fast meat samples were $9.8 \times 10^2 \pm 0.12 \times 10^2$, $1.2 \times 10^3 \pm 0.24 \times 10^2$ and $8.3 \times 10^2 \pm 0.09 \times 10^2$ CFU/g in shawarma, sausage and beef burger, respectively.

Statistically, there are significant differences between shawarma, beef burger and sausage, the later is higher in its *S. aureus* count than the others. These results are nearly agree with El-Daly (1983); Hoshyar, *et al*. (1984). Ayaz, *et al*. (1985) and Morshdy, *et al.* (1986). Lower findings achieved by Soliman (1988), Nassar (1988), Refaie and Moustafa (1990) and Daif (1996), while more than those obtained by El-Sherbeeny *et al.* (1985), Fathi (1988) and Little *et al*. (2001). In this respect, Frazier and Westhaff (1978) reported that the growth of *S.*

aureus in cooked or sterilized food is better than in raw foods if contamination occurs after manufacture and also they reported that the risk of *S. aureus* in burger is relatively low compared to the volume of burger eaten, this may be due to burger is usually cooks well.

Outbreaks could be resulted when processed or cooked meat has been improperly handled and mistreated in food processing plants and/or catering and food service establishments (ICMSF, 1980). Zein El-Abdin *et al*. (1992) reported that the increase in the bacterial counts of meat meals during distribution in aluminum dishes could be attributed to the holding temperature.

The results in Table 3 showed that the highest frequency distribution for shawarma and beef burger lies within the range of 10^2-10^3 *S. aureus*/g., while that of sausage samples was within 10^3-10^4. Wieneke *et al*. (1993) recorded that meat and meat products were the vehicles of 75% of incidents of food poisoning with ham and chickens implicated, in the United Kingdom, (1969-90) and the level of *S. aureus* present in food ranged from no viable cells detected to 1.5 X 10^{10} C.F.U./g. with a median of 3 X 10^7 C.F.U./g. Wamola (1992) stated that the handling and preparation of food when in correct manner can reduce the level of microbial contamination thereby enhancing shelf life and he added that, certain foods carry a higher risk of microbial contamination than others. Little *et al*. (2001) isolated *S. aureus* from ready-to-eat burger with a level of 10^2 CFU/g, while Elmal *et al*. (2005) could detect *S. aureus* in fast beef doner kebabs in counts $<10^2$ to 10^4 CFU/gm. In this respect Fernandez *et al*. (2006) evaluated the effect of storage time and temperature on the microbiological quality of meat burger. They determined *S. aureus* in the burger samples and stated that fast food restaurant must follow the international standards.

As shown in Table 4 the results illustrated that the incidence of enterotoxins produced by *S. aureus* in shawarma was 37.5%, where 3 strains belonged to type A. In sausage, 5 (45.5%) of the recorded strains were enterotoxigenic where 2 of them were type A enterotoxin and the other 3 strains were enterotoxins AD. Four enterotoxigenic strains were obtained from beef burger samples (44.5%) and were identified as 2 type AB and 2 type ABCD staphylococcal enterotoxins. These results agree with those obtained by Adesiyun (1984) who detected enterotoxins from ready-to-eat beef *S. aureus* isolates (43.3%). The author stated that beef isolates of *S. aureus* were the most frequently toxic than other food products and may pose a health hazard with high incidence of enterotoxin type D. Bergdoll (1989) reported that *S. aureus*

causes food poisoning by the production of one or more heat stable extracellular toxins, responsible for the symptoms of the disease, time of onset and severity of symptoms depend on the amount of toxin consumed and individual susceptibility. The enterotoxins produced at detectable levels (> 0.1 ng/gm) in foods occurs only when growth reaches approximately 10^6 CFU/gm (Robbins *et al*., 1974 and Evenson *et al*., 1988).

The summarized results recorded in Table 4 nearly agree with those obtained by Adesiyun (1984); Rosec *et al*. (1997); Holeckova *et al*. (2002); Firinu *et al*. (2003) and Bystron (2005). Lower findings were achieved by Kumar *et al*. (2001) and Manfreda *et al*. (2005). It was found also that enterotoxin type A (SEA) is the most frequently followed by enterotoxin type D (SED) such results go parallel with the results of Minor and Marth (1972); Helena-Lopes *et al*. (1993) and Erol and Iseri (2004), they reported that staphylococcal enterotoxins A (SEA) and D (SED) being the most responsible for the majority of staphylococcus food poisoning. However, there is no relation between the enterotoxigenicity and the other biochemical charcateries (Bystron *et al*., 2002). On the other hand, the direct extraction of staphylococcal enterotoxins from the food samples revealed negative results (not detectable). Halpin-Dohnalek and Marth (1989) stated that enterotoxins can cause food poisoning are produced by about one-third (1/3) of the coagulase positive strains of *S. aureus* and the production of enterotoxigns is affected by the nutritional quality and pH of the substrate, temp., atmosphere, sodium chloride, water activity, other chemicals and competing microorganisms. It is benefit to mention that the reversed passive Latex agglutination assay (RPLA) for staphylococcal enterotoxins detection is a method based on the specific antibodies directed to the enterotoxins and characterized by its accuracy and easy to use. Bergdoll (1990) stated that the detection of *S. aureus* enterotoxins in food requires much more sensitive methods than those required for the determination of enterotoxigenicity of strains, and the quantity of enterotoxin present in foods involved in food poisoning outbreaks vary from less than 1 ng/gm to greater than 1 μg/gm food.

Considering the importance and public health hazard of *S. aureus* organism recovered from fast food (ready-to-eat meat sandwiches), Longree and Blacker (1971) reported that preparing and serving food to the public is a very important obligation that can only be fulfilled if every one in the establishment understand food hygiene, applying sanitary measures at every stage of the operation. Furthermore, ICMSF

(1988) stated that cooked meat should not be touched by hands or by equipments that have come in contact with raw meat equipments that have come in contact with raw meat, raw products should be separated from cooked products to avoid cross-contamination.

To safe the ready-to-eat sandwiches sold in fast food services it must be focus on prevention of contamination and multiplication of microbes and production of toxins. Food should not be prepared long in advance of consumption (Bryan *et al*., 1992). Cooking usually give time temperature exposures that would have been lethal for vegetative form of food borne pathogens. On the other hand, holding of food provide time temperature exposures conductive to microbial growth, particularly in food helds overnight and large populations of aerobic organisms including *S. aureus* and others were recovered from these food. So, time temperature had variable effect of killing the microorganism but heat stable toxins still not affected (Jermini *et al*., 1997; Pepe *et al*., 2006).

In conclusion, it can be achieved from the obtained data that fast meat products (sandwiches) have the potential to cause staphylococcal intoxication to consumers. So, the rules of health agencies must reach to all workers in such field especially street vendors and fast food takeaway restaurants besides safety programs for safe food preparation drawn by WHO (1989) should be followed and effective preventive measures must be authorized and applied to safe the consumer health.

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