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INCIDENCE OF ENTEROTOXIGENIC *STAPHYLOCOCCUS AUREUS* IN SOME READY-TO-EAT MEAT SANDWICHES IN ASSUIT CITY WITH SPECIAL REFERENCE TO METHICILLIN RESISTANT *STAPHYLOCOCUUS AUREUS* STRAINS

(With 4 Tables)

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مدى تواجد المكور العنقودى الذهبى المفرز للسموم في بعض اللحوم المجهزة للأكل في مدينة أسيوط مع إشارة خاصة لعترات المكور العنقودى الذهبى المقاوم للميثيسيللين

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أجريت هذه الدراسة لمعرفة مدى تلوث الوجبات السريعة (ساندويتشات) المجهزة للأكل من منتجات اللحوم (حواوشى ، كبده ، كفته وشاورمه) والمجمعة من محلات الوجبات السريعة بمدينة أسيوط بميكروب المكور العنقودي الذهبي وميكروب المكور العنقودي الذهبي المقاوم للميثيسيللين وكذلك للوقوف على معرفة مدى إفرار العترات المعزولة للسموم المعوية المسببة للتسمم الغذائي. ولقد شملت الدراسة إجراء الفحص البكتريولوجى لعدد 80 عينة حيث أفضت إلى وجود الميكروب المكور العنقودي الذهبي بنسب 25% ، 45% ، 40% و30% في كل من الحواوشى والكبده والكفتة والشاورمه على التوالي. وقد كانت متوسطات عدد الميكروب الكلى هو 2.75×10^3 ، 2.80×10^4 ، 7.20×10^4 و8.98×10^3/ جم فى العينات المفحوصة على التوالي. بينما تم عزل الميكروب المكور العنقودي الذهبي المقاوم للميثيسيللين من جميع أنواع العينات المفحوصة بنسب 15 ، 40 ، 35 و 25 % على الترتيب. وتم تصنيف العترات المعزولة لمدى إفرازها للسموم ولوحظ أن نوع السم C الأكثر وجودا في العترات المعزولة. ولقد نوقشت الأهمية الصحية للميكروب وسمومه ووضعت التوصيات اللازمة لسلامة المستهلك والمنتج.

SUMMARY

A total of 80 random samples of ready-to-eat meat sandwiches represented as 20 each of hawawshy**,** liver, kofta and shawarma that retailed from various fast food restaurants in Assiut city were examined for contamination with *S. aureus* and methicillin-resistant *S. aureus* in association with its enterotoxigenicity. *S. aureus* strains were recovered from 25, 45, 40 and 30% of the examined hawawshy**,** liver, kofta and shawarma samples, respectively. While, the average counts were 2.75×10^3, 2.80×10^4, 7.20×10^4 and 8.98×10^3 cfu/g of the examined samples, respectively. Whereas, MRSA strains were isolated from 15, 40, 35 and 25% of the same examined samples, respectively. Eight out of twelve strains of MRSA were isolated from liver (4 strains), shawarma (3 strains) and only one strain from kofta proved to be enterotoxigenic, while the strains isolated from hawawshy failed to produce any enterotoxins. All the 8 strains produced enterotoxins C, while, 3 strains isolated from shawarma produced CD, ACD and ABCDE enterotoxins, in addition the only strain isolated from kofta can produce CE enterotoxins. The results showed that enterotoxin C was the most frequently in all the examined ready-to-eat sandwiches, indicating that *S. aureus* had a potential public health significance in fast food.

Key words: *S. aureus, MRSA, hawawshy, kofta, liver, shawarma, enterotoxin.*

INTRODUCTION

Nowadays, meat products consumed as sandwiches of shawarma, kofta, hawawshy, etc. are commonly prepared and sold by many restaurants which are widely distributed all over the country (Take-away). *Staphylococcus aureus* (*S. aureus*) is one of the most important microorganisms which can contaminate or recontaminate cooked foods via workers hands, equipments or utensils (Bryan, 1988).

S. aureus is a cluster forming spherical Gram-positive bacterium which is known to cause food-borne intoxication, as some of its pathogenic strains are capable of producing heat-stable enterotoxins. Although this facultative anaerobic bacterium possesses a wide spectrum of virulence properties, including extracellular proteins like adhesions, invasions, hemolysins, extoxins, etc., staphylococcal enterotoxins (SEs) are recognized as the most important factors for its pathogenicity. The production of SE by this bacterium is recognized as one of the predominant food-borne problems causing gastroenteritis worldwide. Contamination by toxigenic *S. aureus* in ready-to-eat food is a major

public health issue in both developing countries like Vietnam and developed countries like the USA, Japan, etc. During 1997, approximately 185,000 people suffered from the SE related food-poisoning including thousand of deaths (Mead *et al.,* 1999).

Staphylococcal food poisoning (SFP) is a mild intoxication occurring after the ingestion of food containing from 20 ng to < 1µg of staphylococcal entertoxin (SE), enough to determine symptoms in human beings (Berdgoll, 1989). SFP symptoms appear within a few hours (i.e. 1-6 h) after ingestion of contaminated food, depending on individual susceptibility and toxic dose ingested. They include nausea, abdominal cramps, diarrhea and a characteristic projectile vomiting (Le Loir *et al.,* 2003).

Lack of proper hygienic measures during food preparation is one of the major sources of contamination as the food-handlers themselves can harbor the pathogenic bacterium. Besides, *S. aureus* can tolerate a wide range of temperature, pH and salinity (Stewart *et al.,* 2002). Proper understanding and extensive knowledge about the routes of *S. aureus* contamination is important for the effective control of related disease outbreaks.

Most of the nosocomial *S. aureus* infections are caused by methicillin-resistant *S. aureus* (MRSA) strains and have become a widely recognized cause of morbidity and mortality throughout the world (Ho *et al.,* 2008). In addition, MRSA strains resistant to quinolones or multiresistant to other antibiotics have been emerging, leaving a limited choice for their control (Pesavento *et al.,* 2007). Furthermore, community acquired MRSA infection has been reported in 2001, when a family was involved in an outbreak from ingestion of MRSA with baked meat, contaminated from the food handler (Jones *et al.,* 2002).

Various ready-to-eat products are becoming increasingly popular in this developing country, particularly in the metropolitan areas. The occurrence and patterns of enterotoxigenic *S. aureus* in ready-to-eat food products has been reported from different parts of the world including South East Asian countries like Taiwan, South Korea, Thailand, etc. (Chomvarin *et al.,* 2006; Oh *et al.,* 2007; Chiang *et al.,* 2008). Therefore, the present work was conducted to investigate the incidence of enterotoxigenic *S. aureus* and methicillin-resistant *S. aureus* (MRSA) strains in different popular ready-to-eat sandwiches (hawawshy**,** kofta, liver and shawarma) in Assuit city as well as to determine the prevalence of the major SEs among the isolated *S. aureus* strains.

MATERIALS and METHODS

1- Collection of samples:

A total of 80 random samples of ready-to-eat sandwiches were collected from different fast food restaurants with different sanitation levels in Assuit City. Sandwiches types evaluated were hawawshy, kofta, liver and shawarma (20 of each). All samples were directly transferred to the laboratory in an ice box under hygienic conditions without delay to be examined bacteriologically.

2- Preparation of samples: (APHA, 1992)

Ten grams of each meat product sample only without bread were homogenized aseptically for 1 min with 90 ml of 0.1% peptone water in a stomacher (Colworth, 400). It was then serially diluted 10-fold in the same diluent.

3- Determination of *S. aureus* count: (AOAC, 2000)

0.1ml from each of the prepared dilutions was spread onto duplicate plates of Baird-Parker (BP) agar (Oxoid CM 275), supplemented with egg yolk tellurite emulsion (50 ml/L, Oxoid SR54) and incubated at 37°C for 24-48h. Colonies with typical *S. aureus* morphology (i.e., circular, black, shiny with narrow white margins and surrounded by clear zones extending into the opaque medium) were counted and recorded.

4- Isolation of *S. aureus*:

Enrichment procedures: (Lee, 2003)

Ten grams of each meat product samples were inoculated into 100 ml of staphylococcus broth (Difco, 264920) and incubated at 35°C for 20h with shaking.

Selective plating:

A loopful from the incubated broth was streaked onto Baird-Parker agar (Thatcher and Clarck, 1975) and incubated at 37°C for 24h. Suspected colonies were subcultured on slants of Brain Heart Infusion (BHI) agar (Oxoid CM225) and incubated at 37 °C for 24h before being subjected to identification.

Identification of isolates:

Isolated purified strains were identified morphologically by Gram's stain and biochemically confirmed as *S. aureus* according to FDA (2001) by the conventional methods that included catalase, production of coagulase and anaerobic utilization of glucose and mannitol.

5- Isolation of MRSA: (Simor *et al.*, 2001)

The isolated strains of *S. aureus* were streaked onto Oxacillin Resistance Screening Agar Base (ORSAB) (Oxoid, CM1008) supplemented with ORSAB selective supplement (Oxoid, SR0195). The plates were incubated at 37°C for 24-48h and examined for the presence of blue colonies.

6- Detection of staphylococcal enterotoxins: (Park *et al.*, 1994)

Production of enterotoxins A, B, C, D and E was determined by a RIDASCREEN kit (R- Biopharm, R4101) according to the manufacturer's instructions. A colony of MRSA was incubated in Brain Heart Infusion broth (Oxoid, CM1032) for 12h at 37°C. The culture was centrifuged and the supernatants were tested for enterotoxin production.

RESULTS

The obtained results are recorded in Tables 1-4

Table 1: Statistical values of *S. aureus* count/g of the examined ready-to-eat sandwiches (No. =20 of each)

Examined samples	Positive samples		Min.	Max.	Average
	No.	%			
Hawawshy	4	20.0	5×10^2	8.7×10^3	2.75×10^2
Liver	6	30.0	7×10^2	8.6×10^4	2.8×10^4
Kofta	7	35.0	7×10^2	2×10^5	7.2×10^4
Shawarma	5	25.0	41×0^2	3×10^4	8.98×10^3

Table 2: Incidence of *S. aureus* in the examined ready-to-eat sandwiches

Examined samples	No. of examined samples	Positive samples	
		No.	%
Hawawshy	20	5	25.0
Liver	20	9	45.0
Kofta	20	8	40.0
Shawarma	20	6	30.0
Total	80	28	35.0

Table 3: Incidence of MRSA strains isolated from the examined ready-to-eat sandwiches

Examined samples	Number of examined samples	Positive samples	
		No.	%
Hawawshy	20	3	15.0
Liver	20	8	40.0
Kofta	20	7	35.0
Shawarma	20	5	25.0
Total	80	23	28.75

Table 4: Distribution of multiple enterotoxins produced by some strains of *S. aureus* isolated from ready-to-eat sandwiches

Product	No.of strains tested	No. of strains producing enterotoxins	Types of produced enterotoxins					
			C	CD	CE	ACD	ACE	ABCDE
Hawawshy	2	0	-	-	-	-	-	-
Liver	4	4	3	-	-	-	1	-
Kofta	3	1	-	-	1	-	-	-
Shawarma	3	3	-	1	-	1	-	1

DISCUSSION

The results recorded in Table 1 revealed that the average counts of *S. aureus* in the examined ready-to-eat meat sandwiches were 2.75×10^3, 2.8×10^4, $7.2\times10^4$and 8.98×10^3 cfu/g in hawawshy, liver, kofta and shawarma, respectively.

The four ready-to-eat products whose staphylococcal isolates were investigated were found earlier to be highly contaminated with staphylococci. Aycicek *et al.* (2005) reported that meatballs and liver samples contained *S. aureus* with counts ranging from 3.7-4.1 and 2.5-3.6 log cfu/g, respectively. In Egypt, EL-Mossalami *et al.* (2008) mentioned that shawarma and liver sandwiches have *S. aureus* with counts ranged from 3.4×10^2 to 5.2×10^4 and 3.7×10^2 to 6×10^4 cfu/g, respectively. While, Shalaby and Zaki (2008) could detect *S. aureus* in shawarma in numbers varied from 2×10^2 to 3×10^3 with a mean value of 9.8×10^2 cfu/g.

According to the US Food and Drug Administration (http://www.cfsan.fda.gov/~mow/ chap3.html.Food), $\geq 10^5$ CFU/g *S. aureus* is capable of causing staphylococcal intoxication. A simulation model of risk assessment has shown that only few cells in ready-to-eat food can have 3–4 log increases at ambient temperature within 5 h (Rho and Schaffner, 2007). The number of *S. aureus* population in the observed ready-to-eat food samples are lower than the required dose to induce food-borne illness but some foods can be considered to have potential risk as there are chances of the bacterium's multiplication during the time of food poisoning as well as if these foods are kept at room temperature for long time in tropical climate.

Out of 80 ready-to-eat samples examined, 28 (35.0%) were found to be contaminated with S. *aureus* (Table 2). Liver samples showed the highest prevalence (9 out of 20; 45%) of S. *aureus* contamination. The bacterium was detected in 25, 40 and 30% of hawawshy, kofta and shawarma sandwiches, respectively.

Thus our study revealed a comparatively higher prevalence of *S. aureus* in the examined samples. However, the ready-to-eat food items possessed risk of contamination as they were exclusively prepared by small-scale local producers without quality control checking for bacterial pathogens. Improper handling and possible cross-contamination during transportation and storage is also possible. Besides, re-used or improperly washed containers or equipment and primary packaging can also be sources of contamination. A recent study in Botswana reported that 57.5% of the food handlers harbored *S. aureus* bacterium and 21% of them possessed toxigenic strains (Loeto *et al.,* 2007).

Regarding the incidence of *S. aureus* recorded in Table 2, the obtained results were less than those obtained by EL-Mossalami *et al.* (2008) who isolated the organism from 22 (88%) and 20 (80%) of ready-to-eat shawarma and liver sandwiches, respectively, and higher than those obtained by Soriano *et al.* (2002) who detected *S. aureus* in 16.9% of meatballs samples and Aycicek *et al.* (2005) who isolated the bacterium from 17 (11.8%) and 3 (9.4%) of meatballs and liver sandwiches, respectively. Moreover, similar results recorded by Shalaby and Zaki (2008) who reported that 32% of the examined shawarma samples were contaminated with *S. aureus.*

According to our results, a high frequency of methicillin resistance was encountered for *S. aureus* strains isolated from liver (40%), kofta (35%), shawarma (25%) and hawawshy (15%) (Table 3).

The results of the present study highlighted that; these foods may constitute a risk for consumers and especially for immunocompromised individuals. In immunocompromised persons the specific and non-specific immune responses are not able to act as barriers to prevent colonization of the gastrointestinal tract and ingestion of food contaminated by MRSA may lead to sometimes lethal disease (Kluytmans *et al.*, 1995).

SFP is one of the most common causes of food-borne illness due to the widespread occurrence of *S. aureus* and to the ability of many strains to synthesize one or more SEs. Inspection of Table 4 revealed that out of 12 methicillin resistance *S. aureus* strains tested for enterotoxins production 8 strains possessed the targeted classical SEs. In liver, all four tested strains were enterotoxigenic where 3 of them were type C enterotoxins and one strain produced ACE enterotoxins. Also, all three tested strains in shawarma were enterotoxigenic, 1(CD), 1(ACD) and 1 (ABCDE). One the other side, the strains isolated from hawawshy failed to produce any enterotoxins. Moreover, from the 3 strains of S. *aureus* isolated from kofta only one strain had the ability to produce enterotoxin CE.

The distribution of the types of SE produced is quite different from those reported in the literature because in our material the SEC is predominantly produced. Similar results reported by Normanno *et al.* (2005) who found that most of the isolates isolated produced SEC (33.9%) and Rosec *et al.* (1997) stated that enterotoxin C was produced by 66% of the enterotoxigenic strains, singly or in combination with other enterotoxins.

Considering the importance and public health hazard of *S. aureus* organism recovered from fast food (ready-to-eat 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; 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 hands, holding of food provide time temperature exposures conductive to microbial growth, particularly in food holds 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 and 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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