

## Isolation of Enterotoxin- Producing *S. aureus* from Hospital Food Handlers, Makkah, Saudi Arabia

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### Abstract:

*A study on enterotoxin-producing Staphylococcus aureus was carried out among food handlers in hospital-located kitchens in Makkah, Saudi Arabia to detect the enterotoxigenic S. aureus in specimens taken from noses, fingernails, throats and stool of food handlers and to identify susceptibility of S. aureus to certain antibiotics. The study group included 356 food handlers from different nationalities. About 1424 samples were collected, cultured and examined in addition to performance of Sensitivity test. Only 15(1.05% of specimens) were enterotoxigenic S. aureus positive. Most of them (13 specimens) were isolated from nasal swabs, one from nail and the other from throat while all the stool samples were negatives. About 23.08% of nasal Staphylococcus aureus produced enterotoxin A, 30.77% produced enterotoxin B, 15.38% produced C, 15.38% produced D, and 15.38% produced A+C, while snail isolate S. aureus produced enterotoxin A as well as S. aureus from throat. All isolated S. aureus 15(100%) were resistant to Penicillin, 1(6.67%) to Augmentin, 1(6.67%) to Clindamycin, 2(13.33%) to Cefoxitin, 15(100%) to Metronidazole, 13(86.67%) to Piperacillin and 1(6.67%) to Imipenem. In contrast, 14(93.33%) were sensitive to Augmentin, 14(93.33%) to Clindamycin, 12(80%) to Cefoxitin and 2(13.33%) to Piperacillin. It was found that only 1(6.67%) was highly sensitive to Cefoxitin. Food handlers in public food premises who are harbouring Staphylococcus aureus may represent potential risk result in transmission of food poisoning.*

**Key words:** enterotoxin, *Staphylococcus*, food handlers, Makkah, *S. aureus*, antibiotics.

## Introduction

*Staphylococcus aureus* is a common bacteria found usually in the noses, eye, throats and skin of people and animals. These bacteria are present in persons with skin, eye, nose, or throat infections and also in healthy individuals. *Staphylococcus* can cause food poisoning if food, contaminated by food handlers, is ingested.

Food-borne diseases are spread throughout the world. The World Health Organization (WHO) estimated that in developed countries, up to 30% of the population suffer from food borne diseases each year, whereas in developing countries up to 2 million deaths are estimated per year (Mulat et al. 2012). Food poisoning that caused by *Staphylococcus aureus* is one of the most common food-borne diseases, results from ingestion of one or more staphylococcal enterotoxins (SEs) in foods (Argudín et al. 2012). *Staphylococcus aureus* is a Gram-positive bacteria which is a major human pathogen that produces certain exoproteins that cause various types of disease symptoms. Some *S. aureus* strains produce pyrogenic exotoxins, such as staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 (TSST-1) (Klotz et al, 2003). Some foods offer a suitable growth environment for toxin-producing *Staphylococcus aureus*, these foods are milk products, mixed foods, meat and meat products, egg and egg products, cakes and ice cream etc (Jenny et al. 2011).

Food-handlers with poor personal hygiene working in food-service establishments could be potential sources of infection due to pathogenic organisms. One of these common pathogens is *Staphylococcus aureus* (Gashaw et al. 2008).

Enterotoxigenic strains of *staphylococcus aureus* responsible of a type of food poisoning. It accounts for 14–20%

in the USA. In a study conducted in Makkah, 22.4% of food handlers were carriers for *S. aureus*, 20.6% of them were enterotoxigenic *S. aureus* (Atif et al. 2006). This organism may exist on food handler's nose or skin, from which it may be transmitted to foods, and become intoxication agent, if these foods are kept without refrigerating or freezing (Mulat et al. 2012). The presence of *staphylococcus aureus* in nose, throat and mouth is considered a potential risk of infection.

## **Materials and Methods**

### **Food handler's specimens**

Clinical specimens (1424) were collected from 356 food handlers (330 males and 26 females) of different nationalities, whom applied to work in hospital-located kitchens in Makkah. The specimens, which included throat swabs (356), nasal swabs (356), nail swabs (356) and stool samples (356) were examined for presence of *Staphylococcus aureus*.

### **Detection of enterotoxin-producing *Staphylococcus aureus***

Ready-made Amie's medium was used to transport these specimens to the laboratory within five hours. These specimens were sub-cultured directly on 5% sheep blood agar and manitol salt agar. Any morphologically suspected colonies of *Staphylococcus aureus* were examined using coagulase test, and then confirmed by catalase test, Gram staining and DNase test. The detected *Staphylococcus aureus* were cultured individually in Brain Heart Infusion (BHI) at 37° C for 18 – 24, after centrifuging at 3000 rpm at 4° C for 20 min. The supernatant was used for enterotoxin evaluation. The enterotoxins A, B, C and D were detected by using RPLA diagnostic kits.

### **Enterotoxin production in culture medium**

Each isolate of *Staphylococcus aureus* was cultured individually

in Brain Heart Infusion broth (BHI), and incubated at 37°C for 18-20 hrs. Thereafter, the culture was centrifuged at 3000 rpm for 20 minutes, and the supernatant was used in the test (Manufacturer method).

### Sensitivity test

The recommended medium in this test is Mueller Hinton agar, and Kirby-Bauer test method was used. In this study, the control strain (*S. aureus* ATCC-25923) was inoculated on a separate Petri plate medium. The inoculation of the medium's surface with the test organism was made with a cotton swab from broth culture standardized to 0.5 MacFarland. Multi-disks antibiotics were placed on inoculated medium by sterile forceps and each disk was pressed slightly to ensure close contact with the medium. The Plate was then incubated aerobically overnight at 37°C. The radius of the inhibition zones was measured from the edge of the disk to the edge of the inhibition zone. Size of inhibition zones of all isolates were compared with the size of the inhibition zones for *S. aureus* (ATCC-25923).

### Results

Specimen	No of specimens	No of positive specimens	%
Nasal swab	356	13	3.65
Nail swab	356	1	0.28
Throat swab	356	1	0.28
Stool samples	360	0	0.00
Total	1424	15	1.05

**Table 1: The proportion of enterotoxin-producing *Staphylococcus aureus* in food handlers working in hospital-located kitchens in Makkah.**

		Enterotoxin										Total	
		A		B		C		D		A+C			
		No	%	No	%	No	%	No	%	No	%	No	%
S. aureus from	Nose	3	23.08	4	30.77	2	15.38	2	15.38	2	15.38	13	86.67
	Nail	1	100	-	-	-	-	-	-	-	-	1	6.66
	Throat	1	100	-	-	-	-	-	-	-	-	1	6.67
Total		5	33.33	4	2.58	2	13.33	2	13.33	2	13.33	15	100

**Table 2: Types of enterotoxins produced by *Staphylococcus aureus* in food handlers working in hospital-located kitchens in Makkah.**

		Antibiotic													
		PG		AUG		CD		FOX		MZ		PRL		IMI	
		No	%	No	%	No	%	No	%	No	%	No	%	No	%
Reaction	R	15	100	1	06.67	1	06.67	2	13.33	15	100	13	86.67	1	06.67
	S	-	-	14	93.33	14	93.33	12	80.00	-	-	2	13.33	1	06.67
	HS	-	-	-	-	-	-	1	06.67	-	-	-	-	13	86.66
	Total	15	100	15	100	15	100	15	100	15	100	15	100	15	100

**Table 3: Sensitivity of *Staphylococcus aureus* towards certain antibiotics in food handlers working in hospital-located kitchens in Makkah.**

PG=Penicillin AUG= Augmentin CD=Clindamycin FOX= Cefoxitin MZ= Metronidazole PRL= Piperacillin IMI= : Imipenem R=Resistant S=Sensitive H=Highly sensitive

A total of 1424 specimens from 356 food handlers were examined. Only 15(1.05%) were enterotoxigenic *S.aureus* positive. Most of them (13 specimens) were isolated nasal swabs, one from nail and the other from throat while all the stool samples were negatives as shown in table 1. Isolates of *S. aureus* were found to produce enterotoxins A, B, C and D singly or in pairs. The incidence of enterotoxins A, B, C, and D production in isolates of *S. aureus* is presented in Table 2. About 23.08% of nasal *Staphylococcus aureus* produced enterotoxin A, 30.77% produced enterotoxin B, 15.38% produced C, 15.38% produced D, and 15.38% produced A+C, while snail isolate *S.aureus* produced enterotoxin A as well as *S. aureus* from throat. Table illustrated that the isolates of *S. Aureus* exhibited different reactions to some antibiotics i.g. 15(100%) were resistant to Penicillin, 1(6.67%) to Augmentin, 1(6.67%) to Clindamycin, 2(13.33%) to Cefoxitin, 15(100%) to Metronidazole, 13(86.67%) to Piperacillin and 1(6.67%) to Imipenem. In contrast, 14(93.33%) were sensitive to = Augmentin, 14(93.33%) to Clindamycin, 12(80%) to Cefoxitin and 2(13.33%) to Piperacillin. It found that only 1(6.67%) was highly sensitive to Cefoxitin.

## Discussion

*Staphylococcus aureus* can contaminate food by direct contact with carrier body, through skin wounds, respiratory droplets produced when people cough and sneeze. This could be occurred by food handlers when they are preparing or handling foods. The consumption of bacterial contaminated foods may result in food-borne diseases. Such diseases remain a major public health problem globally, particularly in developing countries due to lack of ability to monitor and secure hygienic food handling practices (Ifeadike et al. 2012). Food handlers are considered as reservoir of *Staphylococcus aureus* however the bacterium can present in natural environment i.g. water, air and soil. Many studies isolated such bacteria from restaurant workers e.g. in study conducted among restaurant workers from Kuwait City; it was found that *Staphylococcus aureus* and coagulase-negative *staphylococci* (CNS) were isolated from the hands of food handlers in 50 restaurants in Kuwait City (Udo et al. 1999).

The *S. aureus* enterotoxins (SEs) are potent gastrointestinal exotoxins synthesized by *S. aureus* throughout the logarithmic phase of growth or during the transition from the exponential to the stationary phase (María et al. 2010). In the present study most of enterotoxigenic *S. aureus* were isolated from nose (3.65% of nasal samples). *Staphylococcal* food poisoning resulting from the growth of enterotoxigenic *staphylococci* in foods with the production of enterotoxin is the most common food illness found in almost all parts of the world (Udo et al. 1999). In a study implementad in Makkah, Saudi Arabia, among food handlers in some medical centres, it was found that enterotoxic positive *S. aureus* represented 36% of *S. aureus* isolated from nose of food handlers (Atif et al. 2006).

*Staphylococcus aureus* nasal carriage is a well-defined risk factor of infection with this bacterium (Verhoeven et al. 2010). Only one (0.28%) isolate was observed in nail specimens

as well as in throat while there was no isolates in stool samples. (20.5%) food handlers were positive for nasal carriage of *S. aureus* (Mulat et al. 2012). In a study conducted in Ethiopia, the prevalence of *Staphylococcus aureus* in fingernail specimens was 16.5% (Gashaw et al. 2008). About 7.1% was found in fingernail samples and zero in stool samples of food handlers in the Federal Capital Territory of Nigeria (Atif et al. 2006). SEA, followed by SEB was more frequent, in study carried out by M. A. Arín et al. it was found that SEA, followed by SED, was the enterotoxin most frequently (Argudín et al. 2012). SEB, SEC and SED were found only in nasal specimens. To observe the differences between these isolates and their susceptibility to some antibiotics, the sensitivity test was performed. The results of the Antimicrobial sensitivity test for 15 *S. aureus* isolates producing enterotoxins showed different antibiotic resistance patterns. All isolates were resistant to Penicillin and Metronidazole, while most of them 14(93.33%) were sensitive to Augmentin and Clindamycin. In addition to our results *S. aureus* were found to be resistant to several antibiotics (Kitara et al. 2011). It is a potential risk of spread of drug resistant *staphylococcus aureus* infection.

## **Conclusion**

Food handlers in public food premises who are harbouring *Staphylococcus aureus* may represent potential risk result in transmission of food poisoning. Most of enterotoxigenic *S. aureus* had resistance to certain antibiotics.

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