

Enterotoxigenic *Staphylococcus aureus* amongst Food Handlers in Khartoum State, Sudan

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Abstract:

Background and objectives: Food handlers carrying enterotoxin-producing *Staphylococcus aureus* (*S. aureus*) in their noses or hands can contaminate food, leading to food poisoning. Different methods have been used to detect these toxins; DNA amplification methods can show the presence of enterotoxigenic strains of *S. aureus* before the expression of enterotoxins on the basis of specific gene sequences. The objectives of this study were to assess presence of enterotoxigenic *S. aureus* in nose and hand of food handlers as well as to detect genes responsible for these toxins.

Materials and Methods: Nasal and hand (right and left) swabs were collected from each food handlers participate in this study. The swabs were inoculated on mannitol salt agar plates and incubated aerobically overnight. Identification of *S. aureus* isolates was done by conventional microbiological methods. Detection of genes responsible for production of enterotoxins (A, B and C) was carrying out by PCR.

Results: Out of 165 swabs investigated, 48(29%) were positive for *S. aureus*. Of them 21(43.7%) were nasal isolates, while the rest 27(56.3%) were isolated from hands. Out of the 48 *S. aureus* isolated, 9 (18.7%) were associated with staphylococcal enterotoxin A (SEA) gene, 7(14.5%) was associated with staphylococcal enterotoxin B (SEB) gene

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and there is no isolate associated with staphylococcal enterotoxin C (SEC).

Conclusion: It is concluded that there is high percentage of *S. aureus* carriers among food handlers. The most prevalent enterotoxin gene was A, followed by B.

Key words: Food poisoning, enterotoxin gene, Nasal swab, PCR.

INTRODUCTION

Food borne diseases constitute a true health problem in developed as well as and developing countries. They are resulting from ingestion of contaminated foodstuffs, by many different pathogens, or their preformed toxins, It is estimated that up to 30% of the population in developed countries, suffers from food borne diseases each year [1].

Staphylococcus aureus (*S. aureus*) is a common cause of food borne disease worldwide [2]. Staphylococcal food poisoning has been reported to be as a result of an intoxication of preformed staphylococcal enterotoxin [3]. Food handlers considered the main source of enterotoxigenic *S. aureus*. The organism usually exists in their nasal cavities and hands. Argudin et al (2012) stated that *S. aureus* strain present in the nose often contaminate the back of the hand, fingers and face and so nasal carriers are easy become skin carriers [4]. Saeed and Hamid, (2010) reported that the most common isolated pathogen from nasal swab of food handlers were *S. aureus* [5]. The ingestion of the performed toxin of *S. aureus* in food often leads to the development of food poisoning. The symptoms normally have rapid onset 6 hours or less and may include nausea, vomiting, diarrhoea and abdominal pain [6].

Classically, *S. aureus* enterotoxin (SEs) can be classified into 18 types: A-U (except S, F and T) [6]. These were A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, R and U. The SEs are potent gastrointestinal exotoxins synthesized during the

logarithmic phase of growth [7]. They are active in diluted forms, resistant to heat, low pH and proteolytic enzymes [8]. About 95% of poisoning outbreaks are due to classical SEs A, B, C, D and E [9]. On the other hand, for SEC, SEG, SEI and SEU several variants have also been reported [9, 10]. The amount of SEs required for establishment of typical symptoms of food poisoning is very low, ranging from 20 ng to 1 µg [11], which corresponds to approximately 10⁵ CFU of *S. aureus* per gram of food [8]. These toxins are not restricted to *S. aureus* but also produced by some species of staphylococci, including *S. intermedius*, *S. hyicus*, *S. xylosus* and *S. epidermidis* [12]. The objectives of the present study were to assess enterotoxin-producing *S. aureus* among food handlers working in the cafeterias as well as to detect genes responsible for enterotoxins in isolated *S. aureus*.

MATERIALS AND METHODS

This study was carried out in the period from April to October 2013. Data were collected from different cafeterias (n=10) of Sudan University of Science and Technology in Khartoum State. Food handlers (males and females), aged 15-55 years working in these cafeterias were agreed voluntary to participate in the study. Approval of this study was obtained from University Institutional Review Board; informed consents were signed by food handlers who agreed to have nasal and hand swabs. A pretested structured questionnaire was used for collecting information such as age, gender, servicing years, educational status, status of training and habits of hand washing of each food-handler. Swabs from nasal cavity, hands (right hand and left) and fingernails were collected from each food handler. The collected materials were inoculating on Manitol Salt Agar (MSA) plates and incubated aerobically at 37°C for overnight. Only specimens yielded bacterial growth of

yellow colonies were further proceeded for identification by Gram stain and conventional biochemical tests.

DNA Extraction

DNA was extracted from each isolated *S. aureus*. Briefly, 1.5 ml of Brain heart infusion (BHI) overnight culture at 37°C was centrifuged 5000rpm for 2 minutes. The pellet was re-suspended in 567 µl of TE(10 mM Tris-HCl, pH 8.0, 1 mM EDTA) buffer pH(8), 3 µl of 10%SDS and 3 µl of protinase K to give final concentration of 100 µg /ml protinase k in 0.5% SDS, mixed and incubated for 1hour at 37°C, then 100 µl of 5M NaCl was added and mixed thoroughly then 80 µl of CTAB NaCl added, mixed thoroughly and incubated for 10 minutes at 65°C, the protein was removed by adding approximately equal volume (0.7-0.8 ml) of 1:1 CI (chloroform isoamylalcohol), mixed thoroughly and centrifuged at 5000 rpm for 5 minutes then the supernatant was removed in another microcentrifuge tube and an equal volume of 25:24:1 PCI (phenol chloroform isoamylalcohol) addaed, mixed and centrifuged at 5000rpm for 5 minutes then the supernatant was transferred in another microcenrifuge tube and 0.6 volume of cold ethanol was added to precipitate the DNA then washed by 70% ethanol to seprate any protein then after overnight drying the DNA dissolved in 50 µl of TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) buffer and stored at 4°C [13].

Polymerase Chain Reaction (PCR)

Three sets of primers for genes *entA*, *entB* and *entC2* (Metabion International AG) were used. Sequences and related information [14] were tabulated in table (1).

Table 1 shows sequence of primers for genes *entA*, *entB* and *entC2*, their sizes and annealing temperatures

Gene	Primer Sequence)	Size	Annealing temp.
<i>entA</i>	F GTTATCAATGTGCGGGTGG	102	57°C
	R CGCCACTTTTTTCTCTTCGG		
<i>entB</i>	F GTATGGTGGTGTAAGTACTGAGC	164	57°C
	R CCAAATAGTGACGAGTTAGG		
<i>entC2</i>	F AGATGAAGTAGTTGATGTGTATGG	451	57°C
	R CAACTTTTAGAATCAACCG		

PCR amplification

Intron's Maxime PCR premix kit was used in this study. The PCR reaction mixture was prepared as follows; 2 µl template DNA, 1 µl primer F, 1 µl primer R and 16 µl distilled water. The amplification was done using DNA Thermocycler (PTC-100™). The DNA thermocycler was programmed with initial denaturation 5 minutes at 94°C, 30 cycles with 30s denaturation step at 94°C, 30s annealing step at 57°C and 30s extension step at 72°C and final extension at 72°C for 7minutes, holding step at 4°C until the sample was analyzed. The PCR product were electrophoresed, stained with 2µl ethidium bromide and visualized with UV light transilluminator. *S. aureus* ATCC 29213 was used as positive control for *ent A* gene and distilled water as negative control.

STATISTICS ANALYSES

The data obtained were analyzed and presented using statistical Package for social science (SPSS) computer soft ware version 13.0 for windows. Significance of differences was determined using Chi-square test. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

A total of 165 nasal and hand (right and left) swabs were collected from food handlers (n=55) working in ten (10)

cafeterias. Among them, 51(93%) were males and 4(7%) females. The results indicated clearly the existence of *S. aureus* in nasal cavities 21(43.7%), right hand 17/55(30.9%) and in left hand 10/55(18.1%) of the food handlers. There is insignificant differences between the right and left hand (P value =0.12). The presence of *S. aureus* in nostrils of food handlers was reported by Acco *et al.*, (2003) in Brazil [15] and Loeto *et al.*, (2007) in Botswana [16] as 30%, and 44.6%; respectively. The present results confirmed their findings. However, our finding was found to be higher than that reported by authors in Sudan. Saeed and Hamid, (2010) [5] reported *S. aureus* among food handlers as 21.6%, While Abdalla *et al.*, (1998) [17] reported *S. aureus* among hospital workers was 13.2%. Also higher than studies conducted at Gondar University in Northwest Ethiopia [1] and in Fayoum University in Egypt [18] as (20.5%)and (17.1%) respectively. Nasal carriage rates reported by these several studies vary and the variation has been attributed to the differences in the environmental and personnel hygiene of the study population. It is very important to note that although *S. aureus* can cause severe infections, it may also be as a member of the normal flora of the nasal cavity [1]. If by chance, a food handler carries, an enterotoxin producing *S. aureus* he/she may contaminate the food and causes staphylococcal food poisoning outbreak in the population. Moreover, presence of *S. aureus* in left and right hands of food handlers investigated by many authors. Their findings were almost similar to our results. They reported 36.1% at Fayoum University, Egypt [18]. However, the result of recent study in South Africa reported that 88% of hands of food the handlers were contaminated with *S. aureus* [19], which is higher than our findings. This variation in these studies has been attributed to the differences in the degree of safety measures and hand washing in different geographic regions. The results indicated that there is no significant differences between sociodemographic characteristics like education, sex and age in

relation to *S. aureus* detected in nostrils, right hand and left hand of food handlers (P value=0.642).

The result of PCR showed that out of all 48 *S. aureus* isolated from nasal and hand swabs, Staphylococcal enterotoxin A (SEA) was detected in 9(18.7%), SEB was detected in 7(14.5%) but SEC was not detected in all isolated *S. aureus* and there were 3(6.25%) *S. aureus* isolates carried both SEA and SEB genes. Enterotoxigenic strains in this study commonly detected in hand isolates mainly right hand and less commonly detected in nasal isolates. The most common enterotoxin detected is SEA. This result agrees with study conducted in Turkey 14.3% [20]. But differed from other study conducted in Iran which was 25.3% [21]. SEB in this study is the second enterotoxin detected and that agree with studies conducted in Iran (15.8%) [21] and in Kuwait (12.5%) [22]. But did not agree with study conducted in Turkey in which there is no SEB detected [20]. In this last study also no SEC was detected agree with this study but not agree with studies conducted in Iran and Kuwait in which (9.5%), (23%) [21, 22] respectively of *S. aureus* associated with SEC.

CONCLUSION

It is concluded that there is high prevalence of *S. aureus* among food handlers. The presence of the enterotoxigenic *S. aureus* in the food handlers represents a potential health hazard for consumers. SEA gene was the most prevalent, followed by SEB. Education of food handlers in the importance hand washing is highly recommended. Further studies are required to investigate other enterotoxin genes.

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COMPETING INTERESTS

The authors declare that they have no competing of interests.

AUTHORS' CONTRIBUTIONS

HAS, designed, supervised all stages of the study and drafted the paper. AOH, carried out the laboratory procedures, and contributed in drafting the paper. The two authors read and approved the final manuscript.

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Table 1: The sociodemographic characteristics in relation to *S. aureus* detected in nostrils, right hand and left hand of food handlers

Parameter	% of <i>S.aureus</i> in nostrils		AssociationX2 and p value	% of <i>S.aureus</i> in right hand		AssociationX2 and p value	% of <i>S.aureus</i> in left hand		AssociationX2 and p value
	+ve (%)	-ve (%)		+ve (%)	-ve (%)		+ve (%)	-ve (%)	
Education									
Illiterate	2(28.6)	5(71.4)	X2 =0.031 p value =0.882	2(28.6)	5(71.4)	X2 =0.324 p value =0.995	2(28.6)	5(71.4)	X2 =1.676 p value =0.642
Primary school	9(39.2)	14(60.8)		8(34.8)	15(65.2)		5(21.7)	18(78.3)	
Secondary school	9(41)	13(59)		6(27.3)	16(72.7)		3(13.6)	19(86.4)	
Certificate	0(0)	3(100)		1(33.3)	2(66.7)		0(0)	3(100)	
Sex									
Males	18(35.3)	33(64.7)	X2 =0.347 p value =0.556	16(31.4)	35(68.6)	X2 =.071 p value =0.791	9(17.6)	42(82.4)	X2 =.135 p value =0.714
Females	2(50)	2(50)		1(25)	3(75)		1(25)	3(75)	
Age in years									
≤20	9(50)	9(50)	X2 =3.536 p value =0.316	8(44.4)	10(55.6)	X2 =4.117 p value =0.243	5(27.8)	13(72.2)	X2 =5.670 p value =0.129
21-31	5(22.7)	17(77.3)		5(22.7)	17(77.3)		2(9%)	20(91)	
32-41	4(36.4)	7(63.6)		4(36.4)	7(63.6)		1(9)	10(91)	
≥42	2(50)	2(50)		0(0)	4(100)		2(50)	3(50)	

X2 = chi-square

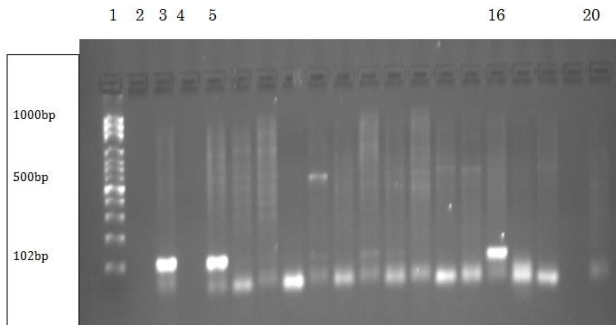


Fig. 1. *S. aureus* shows *entA* genes after PCR on 2% agarose gel electrophoresis lane: 1, 100bp DNA ladder, lane: 2, negative control, lane: 3, positive control, lane: 5, 16 positive *entA* gene.

Figure 1.

- *S. aureus* shows *entA* genes after PCR on 2% agarose gel.
- After PCR and running gel electrophoresis on 2% agarose gel. lane:1, 100bp DNA ladder, lane: 2, negative control, lane: 3, positive control, lane: 5, 16 positive *entA* genes

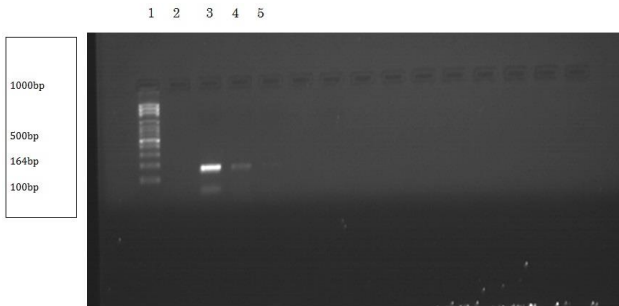


Fig. 2. *S. aureus* shows *entB* genes after PCR on 2% agarose gel electrophoresis lane: 1,100bp DNA ladder, lane: 2, negative control, lane: 3, 4 positive *entB* genes

Figure 2.

- *S. aureus* shows *entB* genes after PCR on 2% agarose gel.
- After PCR and running gel electrophoresis on 2% agarose gel. lane: 1,100bp DNA ladder, lane: 2, negative control, lane: 3, 4 positive *entB* genes.