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# Nasal carriage of enterotoxigenic *Staphylococcus aureus* among food handlers in Kerbala city

Hiba Mahdi Mahmood Alhashimi [a,*], Mohanad Mohsin Ahmed [b], Jassim Mohamed Mustafa [a]

[a] *Department of Biology, College of Sciences, University of Kerbala, Kerbala, Iraq*
[b] *Department of Microbiology, College of Medicine, University of Kerbala, Kerbala, Iraq*

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**Abstract**

**Background:** Staphylococcal Food Poisoning (SFP) is an intoxication caused by consumption of improperly prepared or stored foods containing adequate amounts of one (or more) preformed enterotoxin. Previous studies demonstrated that employees who working in the food industry are the main source for spreading foodborne diseases.
**Aims:** To study the prevalence of the staphylococcal enterotoxin genes among the isolates from the food handlers in Holy Kerbala city.
**Materials and methods:** Nasal swabs were collected from 332 food handlers. Standard microbiological methods were used to isolate *Staphylococcus aureus* including, culturing on selective media (mannitol salt agar), coagulase test and API-staph. Multiplex PCR (Polymerase Chain Reaction) using specific primers was used for the detection of staphylococcal enterotoxin genes.
**Result and discussion:** 100 food handlers out of 332 (30.1%) were found to carry *S. aureus*, 38 (38%) isolates were found positive in Multiplex PCR for one or more enterotoxin: 16 (16%) were positive for *sea*, 18 (18%) were positive for *seb*, 8 (8%) were positive for *sec*, 6 (6%) were positive for *sed* and 8 (8%) were positive for *see*.
**Conclusion:** The prevalence of nasal carriage of *S. aureus* is high among food handlers in Holy Kerbala city, as well as the percentage of enterotoxin genes among the *S. aureus* isolates. Therefore, strict measures are necessary to prevent contamination of food with *S. aureus* isolates during food handling.


*Keywords:* Food poisoning; *Staphylococcus aureus*; Enterotoxin

## 1. Introduction

*Staphylococcus aureus* is Gram-positive bacterium belonging to the family Staphylococcaceae and is often found as a commensal on the skin, skin glands and mucous membranes particularly in the nose of healthy individuals [1].

There is a large number of carriers (more than 30–50% of the population), with *S. aureus* carried persistently or temporarily in human nasal microbiota, without causing any symptoms. The presence of these bacteria in food occurs frequently due to inappropriate manipulation of food by carriers of this microorganism [2].

* Corresponding author.
*E-mail address:* hebamahdiee@gmail.com (H.M.M. Alhashimi).
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Staphylococcal Food Poisoning (SFP) is an intoxication caused by consumption of improperly prepared or stored foods containing adequate amounts of one (or more) preformed enterotoxin [3,4].

Staphylococcal intoxication is often associated with the kinds of foods that include poultry and egg products, meat and meat products, milk and dairy products, bakery products, especially cream-filled pastries and cakes, sandwich fillings and salads [5].

*S. aureus* is able to grow at relatively low water activity (aw = 0.86) so it has been implicated in food poisoning associated with consumption of salted food products [5], though the contamination of food or one of its ingredients during handling. There are several epidemiological features that create the ideal conditions for an outbreak of SFP which include: storage at unsuitable temperatures and the capacity of the microorganism to develop in a wide range of pH, free water concentrations, and sodium chloride concentrations [6].

Previous studies demonstrated that employees working in the food industry are the main source for spreading foodborne diseases [7,8].

Food handlers have been implicated in a plethora of foodborne diseases, and it has been reported that one of the important pathogens often transmitted via food contaminated by infected food handlers is *S. aureus* [9,10]. Nasal and hand carriage of enterotoxigenic *S. aureus* by food handlers is an important source of staphylococcal food contamination in restaurants and fast food outlets, therefore it is important to detect asymptomatic *S. aureus* carriers among food handlers to prevent possible food contamination by them resulting in food poisoning [11,12].

Genes encoding for staphylococcal enterotoxins (SE) have different genetic supports, most of which are mobile genetic elements. *Sea* gene, composed of 771 base pairs, encodes an enterotoxin A precursor of 257 amino acid residues and is carried by a family of temperate phages. *Seb* is an open reading frame encoding the enterotoxin B precursor that consisted of 266 amino acids and is chromosomally located in some clinical isolates, whereas it has been found in a 750 kb plasmid in other *S. aureus* strains. Enterotoxin C is encoded by a gene located on a pathogenicity island containing a 798-base-pair open reading frame that encodes a protein of 266 amino acid residues. *Sed* is located on a plasmid and see is carried by a defective phage. The main regulatory system controlling the gene expression of virulence factors in *S. aureus* is the accessory gene regulator that acts in combination with the staphylococcal accessory regulator. Not all of the SE genes are controlled by the agr system: the *seb*, *sec* and *sed* genes were shown to be agr dependent, whereas *sea* and *sej* are agr independent [13].

In Tehran, Iran multiplex PCR was used to detect *sea*, *seb*, *sec*, and *seq* genes in *S. aureus* isolated from nasal carriers. In an attempt to determine the incidence of newly identified enterotoxins in SFP outbreaks in Iran; in this study, 150 *S. aureus* strains were isolated from nasal carriers by using cotton swabs. Of these, 56 strains were positive for classical enterotoxins, *sea*, *seb*, *sec*, and *seq*. Results showed that 24 (25.3%) isolates were associated with the *sea* gene, 15 (15.8%) isolates were associated with the *seb* gene, 9 (9.5%) isolates were associated with the *sec* gene, 8 (8.4%) isolates were associated with the *seq* gene and 39 (41%) of these isolates might have possessed other se genes but which were not see and *sed* (319 genes). Only one of these 95 isolates harbored *sec* and *sea* [14].

Many methods have been developed in order to detect the toxins quickly with specificity and sensitivity which include: an immunoassay single diffusion tube test, polymerase chain reaction (PCR), an enzyme-linked immunosorbent assay (ELISA), a reversed passive latex agglutination assay (RPLA), and the Ouchterlony double diffusion method (ODD) [15,16].

Several factors must be considered when choosing a method for enterotoxin detection, such as sensitivity, specificity, reproducibility, cost, labor, rapidity, convenience, and the number of samples [17].

### 1.1. Aims

To study the prevalence of the staphylococcal enterotoxin genes among the isolates from the food handlers in Holy Kerbala city by using multiplex polymerase chain reaction.

## 2. Materials and methods

### 2.1. Specimen collection

Nasal swabs from food handlers were examined for *S. aureus* isolation; the nasal swabs were collected in collaboration with the Public Health Laboratory belonging to the Health Directorate of Holy Kerbala Province. Nasal swab specimens were obtained by using sterile dry cotton-wool swabs, and both anterior nares (left and right) were swabbed by rubbing the swab four times around the inside of each nostril while

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