

# Identification of nuc nuclease and sea enterotoxin genes in *Staphylococcus aureus* isolates from nasal mucosa of burn hospital staff: a cross-sectional study

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## Abstract

**Background:** *Staphylococcus aureus* is one of the main causes of nosocomial infections. The hospital staff as carriers of *Staphylococcus aureus* have an important role in spreading it among patients. This study aimed to isolate and identify the sea and nuc genes that encode enterotoxin in *Staphylococcus aureus* isolates, isolated from the Nasal mucosa of staff in Sari Burn hospital by PCR method.

**Methods:** A gene nasal mucosa of Sari Burn hospital staff. In this cross-sectional study, a nasal swab of 40 staff of Burn hospital of Sari was collected and isolated. The *S. aureus* was detected by biochemical tests such as Gram stain, catalase, and coagulase. Then nuc and sea genes were identified after the extraction of DNA, by PCR technique and gel electrophoresis with a specific primer.

**Results:** From the 40 strains obtained from nasal carriers, 20 *S. aureus* strains were isolated, and all of them included the nuc gene, while 6 samples included the sea gene. Given that every 20 samples had the nuc gene, therefore this gene is a strong marker for *S. aureus*. Also, the presence of sea genes in some samples suggested the presence of enterotoxin A in hospital staff as a healthy carrier.

**Conclusions:** PCR techniques can be used to detect the genes encoding enterotoxins in *S. aureus*. Given that hospital staff can be carriers of *Staphylococcus aureus* and spread nosocomial infections, therefore identification of the carriers to prevent the spread of infection is essential.

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**Keywords:** Hospital staff, Nuc nuclease gene, PCR, Sea enterotoxin, *Staphylococcus aureus*

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## Introduction

*Staphylococcus aureus* is an opportunistic pathogen that produces numerous exotoxins and is the most common cause of infection in hospitals after *Pseudomonas aeruginosa* [1]. Various studies show that 15%–80% of *Staphylococcus aureus* strains isolated from different sources can produce enterotoxin [1–3]. Among staphylococcal enterotoxins, the sea (enterotoxin A)

type is more resistant to heat and gastrointestinal proteolytic enzymes such as pepsin and trypsin [4].

The nuc gene acts as a marker and also the presence of heat resistant nuclease gene (nuc) is strongly associated with the production of enterotoxin and it can be considered an indicator of infection with enterotoxin producer *Staphylococcus aureus* [5].

There are several methods for detecting toxins in this bacterium, including latex agglutination, ELISA immunochromatography, latex immunoassay, and magnetic immunoassay. In all these methods, it is necessary to provide conditions for the expression of the staphylococcal enterotoxin gene to identify these toxins. Molecular detection methods are also used to identify staphylococcal enterotoxin genes that not only do not require these conditions but are also able to

identify staphylococci that have secreted low levels of the toxin, which cannot be detected by immunological methods [6]. PCR can detect the susceptibility of enterotoxin-producing strains, especially when enterotoxin genes are not expressed due to different conditions. In these cases, it is important to search for *Staphylococcus aureus* strains containing the classic enterotoxin genes SEA-SEE; Because these enterotoxins in very small amounts can also cause severe food poisoning. For this reason, many researchers today use this method to identify strains of *Staphylococcus aureus* that produce enterotoxins [7].

Nosocomial infections are secondary infections that a patient acquires during hospitalization. One of the most common bacteria causing nosocomial infections is *Staphylococcus aureus*, which due to various virulence factors can play an important role in the transmission of seemingly healthy carriers to patients in various departments, including burns and intensive care units [8].

According to the colonization ability of *S. aureus* on the human mucosal membrane, a wide range of hospital infections are associated with this microorganism [9]. *S. aureus* is defined as the second reason for nosocomial infections [10]. Hospital staff nasal carriers are considered the main source of nosocomial infections in patients [11]. Due to the importance of *Staphylococcus aureus* strains in nosocomial infections and the role of enterotoxins as superantigens in various diseases, the present study aimed to isolate and identify the sea and nuc genes that encode enterotoxin in *Staphylococcus aureus* isolates, isolated from the Nasal mucosa of staff in Sari burn hospital by PCR method.

## Materials and methods

In this descriptive cross-sectional study 40 samples were collected from the nasal mucosa of Sari Burn hospital staff by a simple sampling method, and cultured on the nutrient agar medium (Merck, Germany). In the next step, colonies suspected of *Staphylococcus aureus* were tested using gram staining, catalase, oxidase, and coagulase tests. Colonies suspected of *Staphylococcus aureus* were cultured in mannitol salt agar medium (Merck, Germany). After culturing the bacteria in the mannitol salt agar medium, after 24 hours of culturing the bacteria, a colony loop was inoculated into the nutrient broth medium (Merck, Germany). After 24 hours of incubation at 37 °C, glycerol was added to the mixture of bacteria and broth medium in a ratio of 70 to 30. The resulting suspension was transferred to the Eppendorf and stored at -80 °C.

## DNA extraction

DNA extraction was done with DNA extraction Kit (CinnaGene Co., Iran). 1.5 ml of microbial suspensions were centrifuged at 4500 rpm for 4 min. 100 µl of the precipitant was mixed with 400 µl of the lysis solution and vortexed for 15 to 20 seconds. In the next step, 300 µl of the precipitating solution was added to the mixture and mixed for 3 to 5 s, and placed at 20 °C for 20 min. It was then centrifuged at 12,000 rpm for 10 min and gently emptied by inverting the tube and placing it on paper for 2 to 3 s. One ml of wash buffer was added to the resulting precipitate and vortexed for 3–5 s. It was centrifuged at 12,000 rpm for 5 min, then the wash buffer was completely evacuated and placed at 65 °C for 5 min to dry. The precipitate was completely dissolved in 30 µl of the solvent buffer by gentle shaking and placing at 65 °C for 5 min. The insoluble material was centrifuged at 12,000 RPM for 30 s. The supernatant contains pure DNA.

## Identification of sea and nuc nuclease genes by PCR

In the PCR of the sea and nuc nuclease genes, their specific primers were used after blasting in NCBI to ensure their specificity. These primers were provided by Pishgam Company, Iran. Table 1 lists the specifications for the primers used. The amount and volume of materials required for the PCR and temperature program of the Thermal cycler device (BIO-RAD, USA) are mentioned in Table 2.

## Electrophoresis of PCR products

PCR products were electrophoresed using 1 % agarose gel (Merck, Germany). A mixture of 1 λ DNA loading Dye and 5 λ PCR product was loaded in the gel. Electrophoresis was performed in voltage from 120 to 90 v.

## Statistical analysis

SPSS v19 (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.) was used to analyze the data. The frequency of genes is reported as a percentage frequency.

TABLE 1. The specifications for the primers

Name	Sequence (5-3)	Amplitude size
Nuc F	GCGATTGATGGTGATACGGTT	279
Nuc R	AGCCAAGCCTTGACGAACCTAAAGC	279
Sea F	GGAGTTGGATCTTCAAGCAAGAC	86
Sea R	CCCTCTGAACCTTCCCATCAAA	86
<i>Staphylococcus aureus</i> F	AGTTTGATCCTGGCTC	500
<i>Staphylococcus aureus</i> R	CCTACGTATTACCGCGGC	500

**TABLE 2.** Thermal cycling device temperature program, and the amount and concentration of materials required for PCR

Cycles	Steps	Temperature	Time	Materials	Amounts
<b>Staphylococcus aureus</b>					
First step: 1 cycle	Denaturation	94	4 min	10X buffer solution	2.5 ml
Second step: 35 cycles	Denaturation	95	45 s	NTPs (10 mM)	0.5 ml
	Annealing	57	1 min		
	Extension	72	1 min		
Third step: 1 cycle	Extension	72	10 min	Magnesium chloride (50 mM)	0.75 ml
sea gene				Forward primer	1
First step: 1 cycle	Denaturation	95	5 min	Reverse Primer	1
Second step: 35 cycles	Denaturation	94	50 s	Template	5
	Annealing	59.5	40 s		
	Extension	72	50 s		
Third step: 1 cycle	Extension	72	5 min	DNA polymerase	0.2 ml
nuc gene				Double-distilled water	14.05 ml
First step: 1 cycle	Denaturation	95	5 min	Total volume	25 ml
Second step: 37 cycles	Denaturation	94	1 min		
	Annealing	55	40 s		
	Extension	72	1.5 min		
Third step: 1 cycle	Extension	72	1 min		

## Results

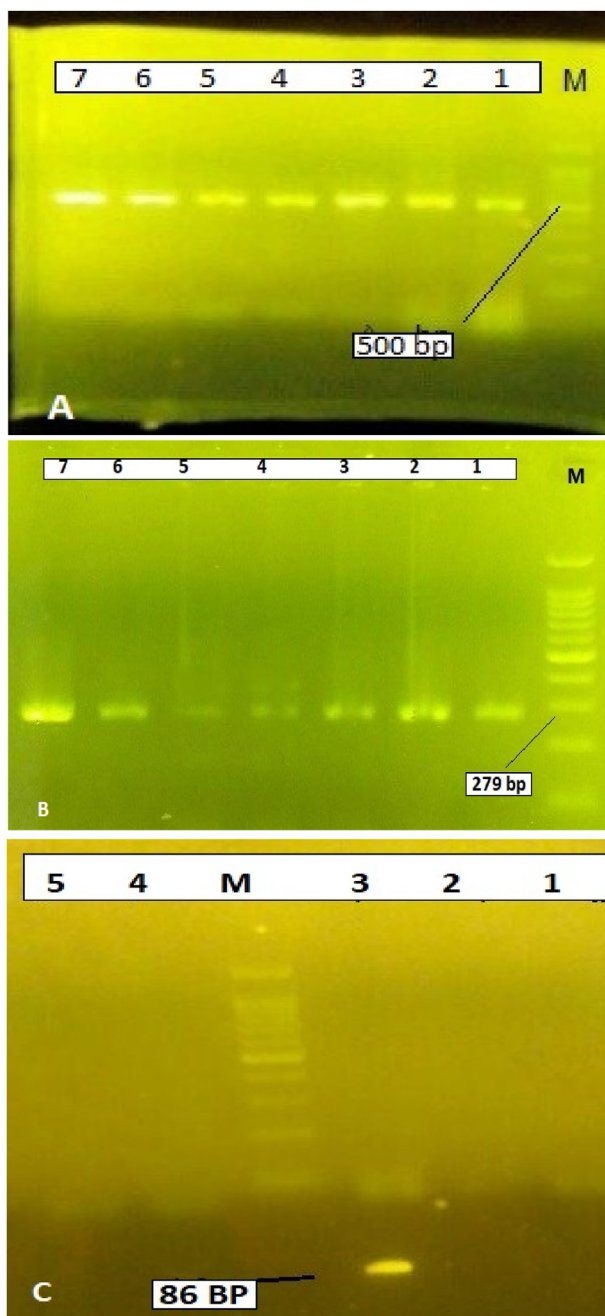
The results of biochemical tests showed that 20 out of 40 samples were positive for *Staphylococcus aureus*. The results of the PCR tests showed that all 20 positive samples had nuc nuclease genes and 6 (30%) of 20 *Staphylococcus aureus* positive samples had sea genes. Fig. 1 shows the results of PCR product electrophoresis.

## Discussion

Because of the significance of *Staphylococcus aureus* strains in nosocomial infections and the role of enterotoxins as superantigens in various diseases, in the present study we evaluated the sea and nuc genes that encode enterotoxin in *Staphylococcus aureus* isolates, isolated from Nasal mucosa of staff in Sari burn hospital by PCR method. According to the results, all 20 positive samples had nuc nuclease genes, and 6 (30%) of 20 *Staphylococcus aureus* positive samples had sea genes.

In a study by Asgarpoor et al. among the 136 nasal swab samples, 46 (33.8%) were positive for *S. aureus*, and from 46 isolates, the sea, seb, and sec genes were found in 11 (23.9%), 6 (13%) and 5 (10.8%) isolates, respectively [12]. In the Rall et al. study 82 nasal and hand, swabs were evaluated in food handlers and found that the most common gene was the sea (35.4%), followed by seh and sej (29.2%) [11]. Amini et al. evaluated the *Staphylococcus aureus* enterotoxin genes using multiplex PCR in 60 clinical samples and showed that 50% of *Staphylococcus aureus* samples contained the enterotoxin gene. The most frequent gene was the sea (30%) and sed (10%), see (3.8%), and sec respectively [13]. Baz et al., evaluated the prevalence of five staphylococcal enterotoxin encoding genes including sea, seb,

sec, sed, and see in isolates from clinical specimens collected from patients admitted to Assiut hospital in Egypt, and using the PCR method found that most isolates (39.1%) were related to enterotoxin A genes [14]. In da Silva et al. work, out of 58 *Staphylococcus* spp. 29 (50%) were positive for one or more enterotoxin genes, and the main genes were seg and sei (29.3%) [15]. In a study by Udo et al. in Kuwait, samples of nasal swabs, feces, and hands of 250 restaurant employees were evaluated to detect staphylococcal enterotoxin genes. From 200 isolates of *Staphylococcus aureus* isolated from people working in restaurants, the frequency of sea, seb, and sec genes were reported to be 11%, 12.5%, and 23%, respectively [16]. Avila-Novoa et al. evaluated the prevalence of the toxin-encoding gene in *S. aureus* isolates from food contact surfaces. They demonstrated that 35.7% of the *S. aureus* isolates had at least one enterotoxin gene. In addition, 8 3.3% of *S. aureus* isolates were positive for amplification of the nuc gene [17]. Peck et al.'s study in Korea showed that the sea gene was the most abundant among enterotoxin genes and out of 95 strains of *Staphylococcus aureus* isolated from the nose of individuals, 47.4% carried this gene [18]. In the study of Saadati et al., from 95 strains of *Staphylococcus aureus* isolated from the noses of carriers, the frequency of sea, seb, and sec genes were 25.3%, 15.8%, and 9.5%, respectively [19]. Mohammad et al. assessed the prevalence of five *s. aureus* enterotoxin encoding genes in isolates from different clinical and environmental sources in hospitals and reported that 66% of enterotoxin gene was sea gene followed by seb (38%), sec (23%), see (19%) and seg (5%) [20]. Diab et al. in a study on 680 milk samples and 43 human hand swabs and 43 human nasal swabs reported that 21.6% of milk samples, 44.2% of the hand swabs, and 32.6% of the nasal swabs were positive for *S. aureus*. Enterotoxin profile genes were found in 64.7% of the milk samples, 63.2% of the hand samples, and 64.3% of the nasal samples. Sea was the most



**FIG. 1.** Electrophoresis of PCR products. A) all samples (1–7) were positive for *Staphylococcus aureus*, M: Marker; B) All samples were positive for *nuc* gene; C) Sample 3 was positive for *sea* gene.

prevalent gene in all sample types [21]. In Goudarzi et al. study, Among 100 isolates from nasal and clinical sources from Khorramabad hospitals, 35% present *sea* gene and 8% *seb* gene [22].

Due to differences in sample size, geographical location, the race of individuals, and level of personal and general health, the

frequency patterns of *Staphylococcus aureus* vectors are different. It seems that *Staphylococcus aureus* strains isolated in the above studies have different enterotoxigenizing power and this important issue is also effective in increasing or decreasing the pathogenicity of this bacterium. It can be said that the location of bacterial colonization can affect the frequency of enterotoxigenic *Staphylococcus aureus*.

Due to the importance of the pathogenicity of *Staphylococcus* bacteria and its role in nosocomial infections in this study, it was found that hospital staff can be considered a source of the spread of infection in the hospital. The identification of enterotoxin A itself acknowledges the important role of these toxins in the development of secondary infections in patients. The high prevalence of the gene encoding this toxin, as well as the expression of this gene in isolates studied in hospitals, can be a warning and a serious threat to public health. Therefore, using the PCR method, the foci of danger can be identified quickly and possible poisoning can be prevented. Controlling the spread of such microorganisms is very important. It is very necessary for the health care system of any society to correctly identify the important and common pathogens in the hospital. Regional studies aimed at obtaining information about the species and type of *Staphylococcus* as well as their resistance can provide physicians with the right solutions in choosing the appropriate treatment guidelines.

It is better to collect data from other hospitals and other cities as well. Other *Staphylococcus aureus* genes should be evaluated as well. Various physicochemical factors that affect the growth and production of *Staphylococcus aureus* toxin have been extensively studied. However, the effect of these physicochemical factors on the genes responsible for the pathogenicity of this bacterium should be studied.

### Ethics approval

The study is performed according to Helsinki's principles of ethics. The ethics committee of Islamic Azad University, Ayatollah Amoli Branch approved the study. All participants signed written consent.

### CRediT author statement

**Roxana Karimzadeh:** Conceptualization, Methodology, Sample collection, Visualization, Investigation and performing the tests; **Romina Karimzadeh Ghassab:** Study design, Data collection and analysis, performing the laboratory tests, Writing- Original draft preparation.

## Conflicts of interest

There is no conflict of interest for the present study.

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