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Original Article

Evaluation of the Frequency of Enterotoxin A (*SEA*) and Enterotoxin B (*SEB*) Genes in Clinical Isolates of *Staphylococcus aureus* in Rafsanjan, Iran

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Abstract

Background: *Staphylococcus aureus* is one of the most important human pathogens that produces a wide range of toxins and causes various diseases. Staphylococcal enterotoxin is the most common cause of food poisoning. In addition, *S. aureus* enterotoxins are classified into 18 serotypes A to U based on serological and biological properties.

Methods: The samples were isolated from clinical specimens and identified by routine bacteriological methods. The isolated *S. aureus* was evaluated by polymerase chain reaction (PCR) for the detection of the genes encoding *SEA* and *SEA*.

Results: Based on the PCR results, 3 isolates possessed the enterotoxins B (*SEB*) gene while none of them showed enterotoxins A (*SEA*) gene.

Conclusions: The obtained results revealed that the clinical samples might be a potential source of the enterotoxigenic strains of *S. aureus*.

Keywords: Enterotoxin A, Enterotoxin B, *Staphylococcus aureus*, PCR

Background

Staphylococcus aureus is an important human pathogen, the virulence potential by which mainly relies on the production of an impressive set of protein toxins (1). This pathogen can work separately or in concert to cause a multitude of human diseases. Pneumonia, sepsis-related infections, toxic shock syndrome, and food poisoning are among the diseases that have traditionally and particularly been associated with the production of enterotoxins (2). In recent years, the prevalence of the bacterium is increasing in hospital-acquired infection and antibiotics prescribed to control and treat the infections have been ineffective due to the resistance phenomenon (3). However, there is no precise mechanism that can explain the invasive diseases caused by *S. aureus* (3), *S. aureus* species produce a variety of exoproteins such as enterotoxins (*SEs*), toxic shock syndrome toxin 1 (TSST1), exfoliative toxins, hemolysin coagulase, and leukocidin. About 15%-81% of *S. aureus* strains are able to produce enterotoxin (4). Enterotoxins are water-soluble proteins, which induce the non-specific T-cell proliferation. These toxins are stable to heat and proteolytic enzymes and could lead to food-borne disease (5). In addition, they stimulate the central nervous system via the toxin action on gastrointestinal nerve receptors and cause nausea, vomiting, and abdominal pain (6). Further, A and B enterotoxins are the most common and important exotoxins in food poisoning and hospital infection (7,8). Furthermore, enterotoxins A (*SEA*) is an important toxin in food poisoning and its gene is carried by the temperate bacteriophage (9). Moreover, enterotoxin B (*SEB*) is an agent in food poisoning and could lead to shortness of breath, widespread systemic damage, and even death in high doses. The *SEB* gene involves up to 900 nucleotides and is located on a chromosome (8). Various techniques are developed for the detection of relevant enterotoxins and genes. Polymerase chain reaction (PCR) is a simple, specific, reliable, and sensitive assay (7). PCR assay is used to determine the enterotoxin gene such as *SEE, SEC, SEA* and *SEB*. Using the molecular methods, the present study aimed to detect the enterotoxins A and B genes

of *S. aureus* in the clinical samples of patients attending Abitaleb hospital in Rafsanjan, Iran.

Materials and Methods

Staphylococcus aureus Isolate Screening and Identification

In this descriptive, cross-sectional study, *S. aureus* isolates were randomly derived from patients who referred to Aliebn Abitaleb hospital, Rafsanjan, Iran in 2017. All samples were cultured in the blood agar and Mueller-Hinton agar media (Merck, Germany) and then incubated at 37° C for 48 hours. In general, 50 isolates of bacteria were screened after some diagnostic tests such as gram-staining, catalase, coagulase, DNase tests, and mannitol fermentation.

DNA Extraction and PCR

DNA was extracted by a DNA extraction kit (Sinaclon, Iran) following the manufacturer's instructions. The quality and quantity of the extracted DNA were evaluated by spectrophotometry and 1% agarose gel electrophoresis. Additionally, PCR was performed for *SEA* and *SEB* genes. The applied primers were evaluated in the BLAST database to confirm the absence of nonspecific binding to the other regions of the genome. The sequences of the primers are shown in Table 1. The PCR reaction was accomplished according to the master mix (Sinaclon, Iran) protocol. The amplification reactions containing 1 µL of the forward primer, 1 µL of the reverse primer, 2 µL $MgCl_2$ (50 mM), 0.5 µL dNTP, 2.5 µL buffers (10×), 2 µL of template DNA, 1 µL of Taq DNA polymerase, and 15 µL of distilled water. The *S. aureus* with *SEA* and *SEB* genes and Master Mix without DNA were considered as positive and negative controls, respectively. In addition, the PCR amplification was performed by the program on a Bio-Rad CFX96 system (Bio-Rad Laboratories Inc., Hercules, CA, USA) and the reaction was performed at 30 cycles (Table 2). The amplicons were analyzed by electrophoresis on 1.0% agarose gel, containing safe stain for 30-40 minutes at 90v, and finally, examined under the ultraviolet (UV) light using a UV light transilluminator (UV Star, Biometra, Germany).

Table 1. The Sequences of Primers

Gene	Primer	Sequences	Product Length	Reference
SEA	F R	5′-TGTATGTATGGAGGTGTAAC-3′ 5′-ATTAACCGAAGGTTCTGT-3′	270 bp	(10)
SEB	F R	5′-TCGCATCAAACTGACAAACG-3′ 5′-GCAGGTACTCTATAAGTGCC-3′	477 bp	(10)

Note. SEA: Enterotoxin A; SEB: Entrotoxin B.

Table 2. Time and Temperature Used in PCR

Number	Steps	Temperature (°C)	Time	Number of Cycles
1	First denaturation	94	30 seconds	1
	Denaturation	94	4 minutes	
2	Annealing	50	30 seconds	30
	Extension	72	1 minute	
3	Final extension	72	30 seconds	1

Note. PCR: Polymerase chain reaction.

Results

After bacteriological diagnostic tests, all 50 isolates were gram-positive, beta-hemolytic, mannitol fermenting, along with catalase and coagulase positive. The *SEA* and *SEB* genes in the isolates of *S. aureus* were determined by the PCR method. The specificity of PCR was performed for positive and negative strains. The 7 *SE*-encoding genes (*SEA* and *SEB*) were detected in the positive strains but not in the negative strains (Figure 1). Furthermore, 270 and 477 bp segments were related to the amplification of a specific fragment of *SEA* and *SEB* genes that are responsible for enterotoxin type A and B and 270 bp for staphylococcal *SEA* gene (Figure 1, lanes 1 and 2), and 477 bp for staphylococcal *SEB* gene (Figure 1, lanes 4, 5, and 6). The result showed that 3 isolates (6%) of the clinical samples contained the *SEB* gene while none of the isolates contained the *SEA* gene (Figure 2).

Discussion

Staphylococci are detectable on the skin and mucous membranes of humans (11). This bacterium might induce allergic inflammatory responses via secreting enterotoxin (*SE*) and could lead to toxic shock syndrome toxin 1 (TSST-1). Humans are the main storage for *S. aureus* in nature. Approximately 30% of healthy people are involved in a bacterium (7,12). In the present study, the presence of enterotoxin A and B was investigated in the clinical samples of patients in the hospital. A total of 6% of the samples contained *SEB* while none of them had *SEA* genes. A study was performed on the strains of *S. aureus* derived from patients who were in the hospital over 5 months and investigated the detection of enterotoxins using the real-time fluorescence PCR and reversed passive latex agglutination (SET-RPLA) methods. Based on the results, the PCR method was more suitable than SET-RPLA in the detection of enterotoxins gene. The results further demonstrated that PCR assay could detect some more enterotoxin genes while SET-RPLA was not able to detect them. Furthermore, it was found that the number of the *SEA* was less than *SEB* in the patient-derived S. *aureus* as shown by our study (7). Similarly, Fluer et al investigated the prevalence of *S. aureus* enterotoxins in the blood and burn wound of the patients by an indirect hemagglutination test and reported that 3% and 76%of the isolated strains were from burn wound and blood generate *SEA*, respectively (13). Moreover, Pourmand et al studied the prevalence of the *SEA* gene in the clinical

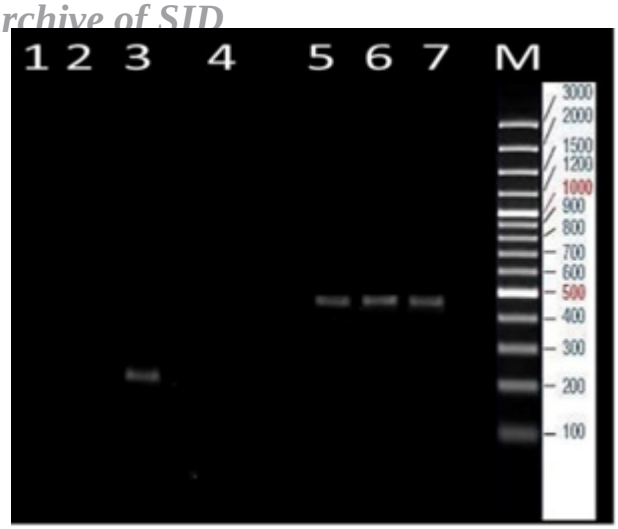

Figure 1. Gel Analysis of PCR-Amplified Toxin Gene Sequences. *Note*. Lanes 1 and 2: *SEA* (270 bp); Lane 3: Positive control *SEA*; Lanes 4, 5, and 6: *SEB* (477 bp); Lane 7: Negative control *SEB*; M: Marker 100 bp.

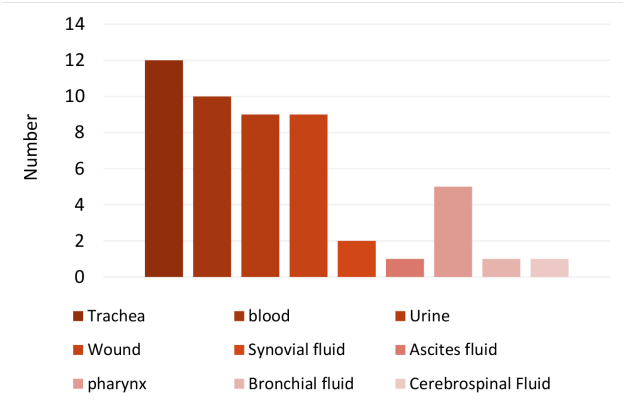

Figure 2. The Distribution of *S. aureus* Isolates in Clinical Samples.

sample of *S. aureus* and showed that 46.9% strains of *S. aureus* contained the *SEA* gene (14). The results of these studies are inconsistent with our results. Another researcher screened the *S. aureus* nasal strains to detect toxin genes and showed that 15% and 13% of the genes were *TST* and *SEC*, respectively. Methicillin-resistant *S. aureus* (MRSA) strains were detected in 4.5% of the students, indicating that the nasal was a perfect site for the growth of MRSA as in our study, bronchial samples contained enterotoxin genes (15). Other investigators defined the rate of enterotoxin gene-positive *S. aureus* isolates among the food handler in central Iran and explained that the rate of *SEA* was more than *SEB* in that food (16). Additionally, Arabestani et al screened *S. aureus* strains from a patient in the west of Iran. They demonstrated that the enterotoxin A gene had the most prevalence in the isolated strains. Their results showed that MRSA strains had the most resistance to cefoxitin and ciprofloxacin while they were completely sensitive to vancomycin (17). In another study, Pourmand et al reported that 98% and 97% of the isolates of *SEA*-positive and *SEA*-negative *S. aureus* were sensitive to vancomycin, respectively (14). Some studies evaluated the enterotoxigenicity of *S. aureus* in dairy products and concluded that these isolates contained a variety of SEs and demonstrated different antimicrobial resistance but almost all of them were resistant to vancomycin (11,18). The results of the present study showed that the prevalence of SE genes is different in a variety of strains as presented in our investigation, the frequency of *SEB* was 6% whereas that of *SEA* was not obvious in any strains.

Conclusions

The obtained results revealed that the clinical samples might be a potential source of the enterotoxigenic strains of *S. aureus*. These findings highlighted the need for strict hygienic and preventive measurements to avoid human health threats.

Conflict of Interest Disclosures

No competing interest was declared by any of the authors.

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