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Original Article

The Relationship between the Presence of Enterotoxin Type B Gene and Antibiotic Resistance in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a hazard to human health since they can cause a wide variety of hospital-associated infections ranging from minor skin infections to post-operative wound infections and food poisoning which produces many different virulence factors, including enterotoxins (SEs). Although studies have been done regarding the difference between virulence factors of sensitive strains and resistant to antibiotics, the aim of this study was to investigate this topic in different patients. This cross-sectional study was performed on 100 patients admitted to a hospital in Tehran. After preparing of wounds samples, antibiogram study was done by disc diffusion method and prevalence of *staphylococcal* enterotoxin type B or *seb* gene was confirmed by Polymerase Chain Reaction. Data was analyzed using SPSS 17 software. Results showed that the highest percentage of isolates with positive *seb* gene is about 7% which related to amoxicillin (6.6%), penicilline and cotrimoxazole (6.5%). More than 90% of isolate are resistant to amoxicillin, penicillin and cotrimoxazol. According to results, a significant relationship between *seb* gene and resistance to related antibiotics was not observed.

Keywords: Antibiotic Resistance, Enterotoxin B Gene, Relationship, *Staphyloccus aureus*

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Introduction

Staphylococcus aureus has been recognized as the main etiological agent and the most frequent microorganism in community-acquired and hospital-acquired infections. During the past four decades, this bacterium has evolved from a controllable nuisance into a serious public health concern. *S. aureus* is a gram-negative bacterium which produces variety virulence factors, including enterotoxins (SEs), which belong to the broad family of pyrogenic toxin super antigens and have emetic activity. Because of fast growth of *S. aureus* in foods and production of entrotoxins by this bacterium, SEs are a common food poisoning. These toxins are a group of heat-stable, pepsin-resistant exotoxins which encoded on phages, pathogenicity islands, chromosomes, or plasmids and belonged to eighteen types including SEA to SEE, SEG to SEQ, SER and SEU [1-3].

Generally, due to special structure, SEs are resistance to high temperature and hence in this conditions that *S. aureus* is not viable, SEs may be present. Among various enterotoxins produced by *S. aureus*, staphylococcal enterotoxin B (SEB) is known via its secretory nature and its potential to binds T-cell receptor and major histocompatibility complex class II. This attachment induce toxic shock mediated by pathological activation of T cells [4, 5].

Based on results from recent studies, SEB is an immunomodulator and that colonization of *S. aureus* may contribute to the pathogenesis of asthma, chronic allergic syndromes, chronic rhinitis, and dermatitis [6]. In recent years multidrug-resistant staphylococci pose a growing problem for human health. Drug-resistant *S. aureus*, especially resistant to β-lactam antibiotics, produces different exo-protein such as enterotoxin, toxic shock toxin, exfoliative toxins, hemolysin, and coagulase. Most clinical studies have not considered the role of microbiological factors such as pathogenic genes in the emergence of pathogenicity of antibiotic resistant *S. aureus*. For example, few studies have been done about the relationship between *Mec* gene in MRSA with PVL (Panton-Valentine leukocidin) on this gene and relationship with other genes related to enter toxin [7, 8].

Considering the increase in antibiotic resistant staphylococcus and large number of reports about Enterotoxigenic strains, investigation the relationship between antibiotic resistant strains and enterotoxin gene is essential. The purpose of this study was to investigate the *seb* gene existence and itsimportance in the emergence of antibiotic resistant strains.

Materials and Methods

Bacteria collection

This cross-sectional study was done in a 14-month period (21 March 2013 till 21 May 2014) on the patients of a military hospital in Tehran. All *Staphylococcus aureus* strains (100 strains) isolated from patients wounds were

collected and investigated. Patients were selected through available sampling method. All *S. aureus* strains underwent the final diagnosis after collection through confirmatory tests, such as mannitol fermentation, coagulase tube and DNAase.

Selection of multidrug-resistant isolates

Agar disk diffusion test was used for screening antibiotic-resistance pattern of isolates in Mueller–Hinton agar (Merck, Germany) using 0.2 ml inoculums (10^8 CFU/ml) of bacteria culture. Specific antibiotic disks (MAST, UK) were selected according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2012, Table 1). After incubation for 24 h at 37°C antibiotic resistant patterns were accomplished following CLSI guidelines. Standard *S. aureus* (ATCC 25923) and Col1 strains were used respectively in antibiogram standardization experiments [9].

Molecular identification of seb gene

Selected strains were analyzed for the presence of the *seb* gene by PCR. For this purpose, DNA extraction was done using Bacteria DNA Gene extraction kit (K-3032, BIONEER Co.; South Korea) and amplification this gene was considered. The reaction mixture consisted of 15 µl 2X master mix (Ampliqon III, Denmark) containing 1.5 mM MgCl2, 1 µl DNA template, 20 pmol of Forward (5'-TCGCATCAAACTGACAAACG-3') and Reverse (5'-GCAGGTACTCTATAAGTGC-3') primers and double distilled sterile water to 30 µl final volume. Also PCR was performed with a thermocycler (Eppendorf) under the following cycling conditions; 95°C for 5 min, then 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s followed by final extension at 72°C for 10 min. Also we were used *S. aureus* conserved *SaCOL* gene as positive control in similar PCR protocol but annealing at 63°C using Forward (5'GCGCGCCATATGTCTGAGCAAGATAACTACGGT T-3') and Reverse (5' GCGCGCCTCGAGGTGAAT-GAAGTTATAACCAGCAG-3') primers. The PCR results were assessed using agarose gel (1%) electrophoresis. DNA ladder SM0333 (Fermentase, Lithuania) was used to determine the size of the amplified fragments [10].

Statistical analysis

The data in each experiment was a representative of three independent experiments expressed as the mean ± standard deviation (SD). The statistical significance of the differences between the control and test values was evaluated using a one-way ANOVA t-test.

Results

Antibiogram test was used to evaluate the antibiotic resistance of bacterial isolates. The inhibitory effect of antibiotics was identified by measuring the inhibition zone diameter of each antibiotic disk according to CLSI standards. Antibiotic-resistance pattern of isolates is summarized in Table 1.

After determination of antibiotic patterns, firstly, we amplified *saCOL* gene (744 bp) as a conserve factor in *S. aureus* by PCR, results showed that amplification of this gene is positive in all strain (Figure 1 & 2). On the other PCR results showed that the highest percentage of isolates with positive *seb* gene is about 7% which related to amoxicillin (6.6%), penicilline and cotrimoxazole (6.5%) (Table 1). Thus, according to these results, a significant relationship between *seb* gene and resistance to related antibiotics was not observed ($p<0.05$).

Table 1. Antibiotic resistance rate of clinical isolates of *Staphylococcus aureus* for each antibiotic and number of isolates that have *SEB* gene.

ANTIBIOTIC	Resistant number (%)	Detected Gen	Percent
AMOXICILLIN	95	7.0	6.6
PENICILLIN	94	7.0	6.5
CO- TRIMOXAZOLE	94	7.0	6.5
OXACILLIN	74	6.0	4.4
TETRACYCLINE	71	6.0	4.2
ERYTHROMYCIN	69	6.0	4.1
DOXYCYCLINE	67	5.0	3.0
CEFTERYACSON	63	4.0	2.5
COLORAMPHENICLE	61	5.0	3.0
CIPROFLOXACIN	56	4.0	2.2
CEPHALEXIN	52	4.0	2.1
VANCOMYCIN	0	0	0

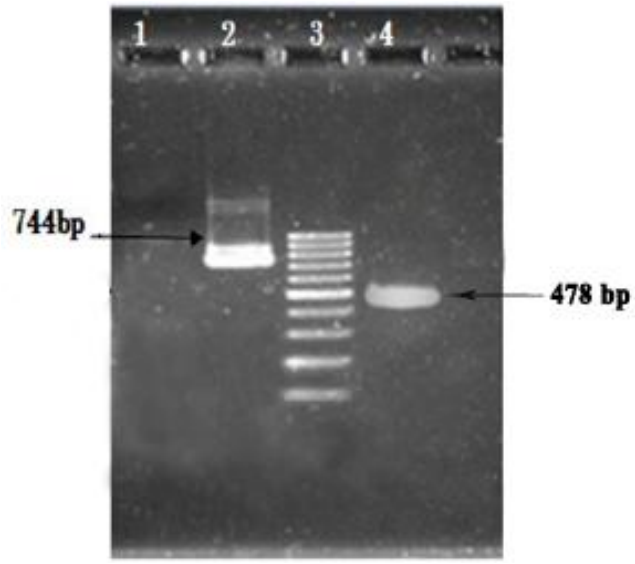

Figure 1. Analysis of the *saCOL* and *seb* gene amplifications from *S. aureus* standard strain on 1% agarose gel. Lane 1: negative control (without template); Lane 2: *seb* amplicon band; Lane 3: DNA size marker (100 bp DNA ladder) and Lane 4: *saCOL* amplicon band.

Discussion

Staphylococcus aureus is so versatile, varied and opportunistic pathogenic bacteria that cause a wide range of infections, ranging from skin to soft tissue infections (such as impetigo, furunculosis, and abscess) to life-threatening pneumonia and toxinoses. The ability of *S. aureus* to cause infection diseases is multifactorial, a

combination of many virulent factors including secreted exoproteins, toxins and cell surface-associated adhesions [11]. *S. aureus* resistance against a wide range of antimicrobials is another important factor in spread of infections by this bacterium that is increasing worldwide, including even resistance to Vancomycin as a main drug choice for *staphylococcal* infections. Generally, for over 50 years Vancomycin has been used for the treatment of *S. aureus* infections, particularly those caused by methicillin-resistant *S. aureus* strains or MRSA that are pervasive in the hospital environment (HA-MRSA) which recently also caused a global epidemic of community-associated *S. aureus* (CA-MRSA) infections [3, 12, 13].

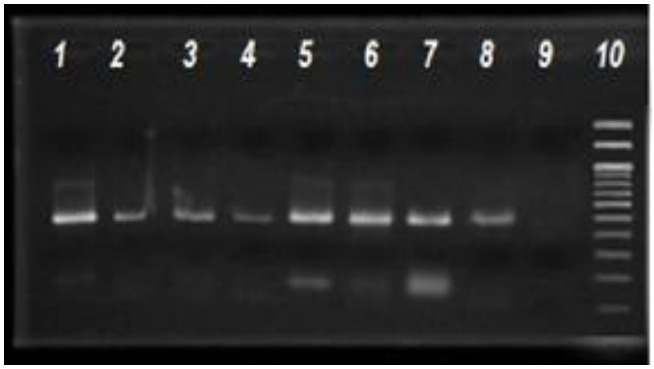

Figure 3. Analysis of *seb* gene amplification from clinical isolates of *S. aureus* on 1% agarose gel. Lane 1-7: *seb* amplicon bands; Lane 8: standard strain; Lane 9: negative control and Lane 10: DNA size marker (100 bp DNA ladder).

However, recently vancomycin-resistant *S. aureus* or VRSA has been reported. Studies have shown that the cause of resistance to all β-lactam antibiotics including methicillin is the *mecA* gene, which is situated on a mobile genetic element that is known as the *staphylococcal* cassette chromosome mec (SCCmec) with seven major variants consist of type I to VII [14]. Based on related studies CA-MRSA harbors SCCmec type IV, V or VII, and these strains are often associated with the presence of the toxin Panton-Valentine leukocidin (PVL) so the presence of resistance genes may also affect toxin production. Accordantly, PVL locus can be used as a suitable marker for detection of CA-MRSA isolates [3, 14].

Accordantly, in our previous study we investigated prevalence of enterotoxin type B gene in meticillin resistant *S. aureus* (n=100) isolated from infection wounds in Tehran. Our results showed only 8% of strains had *SEB* gene. 75% of S. aureus strains that had *SEB* gene were methicillin resistant and only 15% of them were sensitive to methicillin, so a significant correlation was observed between the presence of the *seb* gene and methicillin resistance.

In recent years parallel with our study similar studies have been conducted. For example in 2013, Sina *et al.* [15] investigated variability of antibiotic susceptibility and toxin production of *S. aureus* strains isolated from skin, soft tissue, and bone related infections in Benin. In their study all strains (n=136) were resistant to benzyl penicillin, while 25% were resistant to methicillin and all strains were sensitivity to Vancomycin. Panton-Valentine leukocidin (PVL) was the most commonly produced virulence factor (70%) by strains, while *staphylococcal* enterotoxin B production was 44% and exfoliative toxin B was produced by 1.3% of the strains, and other toxins were rarely detected. Their results showed a high prevalence of PVL-MRSA skin infections in Benin and relationship between PVL and MRSA strains. However results for the *seb* gene was also notable. Also in 2013, Suleiman *et al.* [16], studied enterotoxigenicity and antibiotic resistance of S. aureus (n=103) isolated from sub-clinical bovine mastitis milk in Negiria. Their antibiogram tests on the enterotoxigenic which produced SEA, SEB, SEC and SED and non-enterotoxigenic strains of *S. aureus* showed that there was not a significant relationship between antibiotic resistance and enterotoxin production. The study also revealed high resistance patterns for both enterotoxin (range 20-87%) and non enterotoxin (range 29-69%) strains. On the other hand, Sila *et al.* in a study was compared the prevalence of 13 selected virulence factor genes that included *sea*, *seb*, *seg*, *sei* and *sej* in methicillin-resistant versus methicillin susceptible *S. aureus* (MSSA) isolates.

A total of 200 isolates of *S. aureus* were collected, among them 100 cases were MRSA, the other half were MSSA. Their studies showed the most frequent genes were *seg* and *sei*, coding for enterotoxins G and I (MRSA 77%, MSSA 49%) and the difference in frequency in the two groups was statistically significant ($P<0.05$). It is noteworthy that Enterotoxin B (SEB) had less frequently with 3% in MRSA and 1% in MSSA. Their results about SEB was comparable to detection of seb in 3% of *S. aureus* isolated from blood strains which reported by Becker *et al*. however in compare with our results in previous study (8%) showing lower percentage [17, 18].

Similar to these studies Pereira *et al.* [19] investigated enterotoxin production and antibiotic susceptibility of *S. aureus* isolates from various foods in Portugal. Among 148 coagulase-positive *staphylococcal* strains 69% of the isolates were shown to be enterotoxigenic (SEs) while the most common were SEA/SEG, SEA/SEG/SEI and SEG/SEI. Among these strains 38% of the isolates were resistant to oxacillin but only 0.68% showed the presence of *mecA* gene and also 70 and 73% of the *S. aureus* strains were resistant to beta-lactams, ampicillin and penicillin, respectively.

This study similar to Sila report showed a significant relationship between SEG and SEI producing and antibiotic resistance. Based on these studies in current study for the first time we investigated relationship between SEB present and resistance to common antibiotics which generally used in IRAN. More than 50% of strains were resistance to these antibiotics while less than 7% carried seb gene. Similar to other studies there was not a significant correlation between SEB producing and resistance to common antibiotics, however according to our previous study and other studies correlation between

seb gene present and resistance to antibiotics in MRSA strain is significant. Perhaps this is due to the fact that isolates which have the seb gene are more talented to receive *mecA* gene.

Conclusion

In conclusion our study is the first extensive study for finding relation between SEB and resistance to several common antibiotics in *Staphylococcus aureus* isolated from clinical sample. Our study results show that there are not significant relation between SEB presence and resistance to antibiotics.

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