# High Prevalence of *SEA* Gene Among Clinical Isolates of *Staphylococcus aureus* in Tehran

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**Abstract-** *Staphylococcus aureus* is a major human pathogen producing different types of toxins. Enterotoxin A (*SEA*) is the most common type among clinical and food-related strains. The aim of the present study was to examine the prevalence of *sea* in clinical isolates of *S. aureus*. Moreover, the correlation between *sea* producing strains and type of infection as well as resistance to antibiotics is also considered. 128 *S. aureus* isolates randomly collected from different clinical samples in Tehran University Hospitals from February 2008 to June 2008. Patients' information including sex, infection type and the hospital where samples come from were recorded. The *sea* gene was observed among 60 (46.9%) of clinical isolates. There was a significant correlation between prevalence of *sea* gene and type of infection ($P$ = 0.01). Furthermore, significant correlation was detected between the presence of *sea* gene and resistance to the most of antibiotics used in this study. The significant relationship between the type of infection and *S. aureus* isolates carrying *sea* indicates the interaction quality of the *S. aureus* pathogen and the host as well as the pathogenic role of *S. aureus*.



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**Key words** : *S. aureus*, enterotoxin A, infection

## Introduction

*Staphylococcus aureus* is one of the major human pathogens which can cause various infections like dermal, pulmonary and blood infections. *S. aureus* produces many toxins as well as non-toxic enzymes and can facilitate bacterial attack and proliferation in the body of host (1). *S. aureus* can also produce a series of pyrogenic toxins with superantigenic properties. TSST-1 and enterotoxins are considered to be the most important *S. aureus* superantigens (2) and their role as a prevalent cause of food-related poisoning has been well-defined since many years ago. More than 15 various types of enterotoxins with nearly similar amino acid frequencies have been reported (3). Enterotoxins are heat-resistant proteins which can induce nausea in the central nervous system by stimulating neuron receptors in intestine. However, if the enterotoxins enter the body through a non-oral route (blood, breathing, etc.), they will play their superantigenic role and can stimulate T-cell proliferation, as well (4). Superantigenic effects are fever generation, increased probability of developing toxic shock syndrome and secretion induction in inflammatory cytokines like Tumor Necrosis Factor (TNF-α) and interleukine-1 (5).Staphylococcal enterotoxins Enterotoxins(Ses)are a family of nine major serological types of heat stable enterotoxins. A (SEA) is the most common type in clinical and food related *S. aureus* strains and it has been the incentive for most studies (5, 6). Genes of *sea* and *see* are carried by temperate bacteriophages (7-9). In the meanwhile, *seb* and *sec* are placed on chromosomes and *sed* and *sej* are carried by plasmids (9, 10). Immunologic methods including ELISA, agglutination latex, radioimmunoassay and immunodiffusion were widely used for detecting enterotoxins in sera. Unfortunately, the above-mentioned methods are based on gene expression, so they cannot be of help when the amount of toxin production lessens. Therefore, developing a rapid, sensitive method which can determine the resulting infection seems necessary for detecting toxins within the shortest possible time. In comparison to the very immunologic methods, PCR is preferable considering its higher sensitivity and other features (11-15). The aim of the present study was to examine the prevalence of *sea* in clinical isolates of *S. aureus*. Moreover, the presence of any relationship between enterotoxin A pro-

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ducing strains, type of infection and sex of the patients should also be examined. On the other hand, determining the frequency of the said gene in separated strains of each hospital and medical center as well as the possible relation between *sea* producing isolates and antibiotic resistance will indicate a specific pattern which can determine their differences more clearly.

## Patients and Methods

### Bacterial strains and culture media

This descriptive cross-sectional study was conducted on 128 random *S. aureus* isolates collected from different clinical samples in Tehran University hospitals from February 2008 to June 2008. Patients' information including sex, infection type and the hospital where samples come from were recorded. The isolates were first grown on nutrient agar, manitol salt agar and 5% sheep blood agar at 37°C. After overnight incubation, Gram stain and complementary biochemical tests including coagulase and catalse tests were conducted, as well. Antibiotic susceptibility test was carried out according to NCCLS guidelines. Moreover, we used *S. aureus* ATCC 25923 (*sea* positive) (provided by Dr. Fereshteh Shahcheraghi (Pasteur Institute of Iran, Tehran)) as positive control.

### DNA isolation

The isolates were first subcultured on brain heart infusion agar. Afterward, two loopfuls of cells from overnight subcultures were used for genomic DNA extraction (Bioneer Inc., South Korea). The procedure used for the extraction was similar to that recommended by manufacturer. The concentration of DNA samples was determined as micrograms per milliliter based on $A_{260}$ values and adjusted to 1 µl/ml prior to PCR amplification.

### PCR amplification

Final confirmation of the strains was carried out using PCR with following primers of *S. aureus* coagulase gene(synthesized at TAG Copenhagen, Denmark) as described previously (16). The sequences of the primers were **COA-F** ( 5'-CGA GAC CAA GAT TCA ATA AG-3') and **COA-R** ( 5'-AAA GAA AAC CAC TCA CAT CAG T-3'). Next, PCR reaction was conducted on the final volume of 25 µl using HotStarTaq Master Mix kit (QIAGEN, Germany). In this reaction, 12.5 µl of HotStarTaq Master Mix, 2.5 µl of CoralLoad, 3 µl of the DNA template, 0.5 µl of each primer (20 pM) and 6 µl of $ddH_2O$ mixed. DNA amplification was carried out in a Primus 96 advanced thermocycler (PEQLAB Biotechnologie GmbH, Erlangen, Germany) with the following thermal cycling profile: an initial denaturation at 94 °C for 5 min was followed by 30 cycles of amplification (denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min), ending with a final extension at 72 °C for 10 min. The amplified products were shown by electrophoresis on 1% gel agarose containing ethidium bromide. After final confirmation of the isolates, the following pair of primers was designed and used for the PCR reaction of the enterotoxin A gene: **SEA-F** (5'-TTG CGT AAA AAG TCT GAA TT-3') and **SEA-R** ( 5'-ATT AAC CGA AGG TTC TGT AGA-3'). PCR reaction was conducted like the previous test. The presence of 552bp amplicon indicates the positiveness of *sea* in isolates.

### Validation of sea amplicons

The sizes of *sea* amplicons were similar to those anticipated from the design of the primers. Nevertheless, the resulting amplicons were cut using *Hin*1II restriction enzyme (Fermentas) with cleavage site within the amplicon. The presence of the resulting fragments with the size of 246,154 and 137bp validate the amplified products (results not shown).

### Statistical analysis

Fisher's exact test was used for the statistical analysis of the gathered data. *P* values below 0.05 were considered statistically significant.

## Results

In the present study, 128 *S. aureus* clinical isolates were collected from patients referred to Tehran University hospitals (Shariati (24.2%), Sina (20.3%), Imam Khomeini (17.2%), Loghman (14.8%) hospitals and Children's Medical Center (23.5%)) from February 2008 to June 2008. The PCR results showed that 60 isolates (46.9%) were carrying *sea* (Figure 1). With regards to the hospitals, Shariati had the highest number (17 isolates) of positive *S. aureus* isolates. Sina hospital, Children's Medical Center, Imam Khomeini and Loghman hospitals had 14, 12, 11 and 6 positive cases, respectively. There were 36 females (28.1%) and 92 males (71.8%) in the study. The number of *sea* positive cases in males and females was 47 (51.1%) and 13 (36.1%), respectively. Besides, the prevalence of *sea* positive isolates was not associated with sex or the hospital where samples came from ($P > 0.05$). However, statistical analysis of the data using Fisher's exact test revealed that the prevalence of *S. aureus* isolates carrying *sea*

depend on the type of infection ($P < 0.01$). More interestingly, siginificant correlation was detected between the presence of *sea* gene and resistance to the most of antibiotics used in this study (Tables 1 and 2).

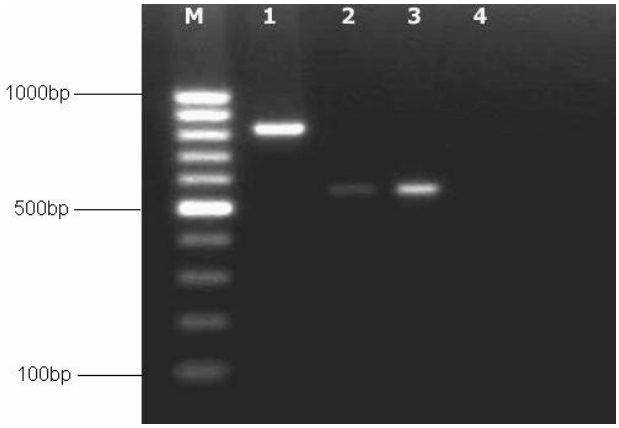


**Figure 1.** 1% gel Agarose electrophoresis patterns showing PCR products from clinical *S. aureus* isolates. Lane M, DNA molecular size marker (100bp ladder; Fermentas Gene Ruler).Lane 1, PCR amplicons from *S. aureus* coagulase gene. Lane 2, *sea* positive control (ATCC 25923). Lane 3, *sea* positive isolate. Lane 4, negative control.

**Table 1.** Percentage of *S.aureus* isolates carrying *sea* gene based on type of infection by trust with 95% CI (Confidence interval) , total CI = 38.1%-55.6%

| Type of infection | *sea* positive, n=60 (%) | 95% CI of *sea* positive | Total, n =128 (%) |
|---|---|---|---|
| Urinary | 7(43.7%) | 19.7%- 70.1% | 16 (12.5%*) |
| Pulmonary | 23(71.9%) | 53.2% - 86.2% | 32 (25%) |
| Dermal | 15 (39.5%) | 24.0%– 56.6% | 38 (29.7%) |
| Blood | 8 (28.6%) | 13.2% - 48.7% | 28 (21.9%) |
| CSF | 2( 40%) | 5.3% – 85.3% | 5 (3.9%) |
| Eye | 2(33.3%) | 4.3%– 77.7% | 6 (4.7%) |
| Bone | 3(100%) | 29.2% – 100%** | 3 (2.3%) |

* Percentage of corresponding sample from total.

** One-sided Fisher's exact test, 97.5% CI.

**Table 2.** Correlation between antibiotic resistance patterns and presence of *sea* gene among clinical isolates of *S.aureus*

| Antibiotic (µg/disc) | *sea* negative | | | *sea* positive | | | *P** |
|---|---|---|---|---|---|---|---|
| | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) | |
| Clindamycin (2µg) | 52 (76.5%) | 0 (0%) | 16 (23.5%) | 27 (45%) | 1 (1.7%) | 32 (53.3%) | *<0.0001* |
| Rifampin (5µg) | 55 (80.9%) | 1 (1.5%) | 12 (17.6%) | 47 (78.3%) | 0 (0%) | 13 (21.7%) | *0.82* |
| Tobramycin (10µg) | 42 (61.8%) | 2 (2.9%) | 24 (35.3%) | 6 (10.0%) | 0 (0%) | 54 (90.0%) | *<0.0001* |
| Methicillin (5µg) | 42 (61.8%) | 0 (0%) | 26 (38.2%) | 7 (11.7%) | 1 (1.7%) | 52 (86.6%) | *<0.0001* |
| Ceftriaxon (30µg) | 39 (57.4%) | 0 (0%) | 29 (42.6%) | 10 (16.7%) | 0 (0%) | 50 (83.3%) | *<0.0001* |
| Ciprofloxacin (5µ) | 43 (63.2%) | 2 (2.9%) | 23 (33.8%) | 11 (18.3%) | 0 (0%) | 49 (81.7%) | *<0.0001* |
| Chloramphenicol (30µ) | 61 (89.7%) | 5 (7.4%) | 2 (2.9%) | 54 (90.0%) | 6 (10.0%) | 0 (0%) | *0.53* |
| Oxacillin (1µg) | 41 (60.3%) | 0 (0%) | 27 (39.7%) | 7 (11.7%) | 0 (0%) | 53 (88.3%) | *<0.0001* |
| Trimethoprim-sulfamethoxazole (1.25/23.75µg) | 46 (67.6%) | 1 (1.5%) | 21 (30.9%) | 18 (30.0%) | 0 (0.0%) | 42 (70.0%) | *<0.0001* |
| Gentamicin (10µg) | 46 (68.7%) | 2 (1.5%) | 20 (29.8%) | 11 (18.3%) | 0 (0%) | 49 (81.7%) | *<0.0001* |
| Tetracycline (30µg) | 36 (53.0%) | 2 (2.9%) | 30 (44.1%) | 8 (13.3%) | 0 (0.0%) | 52 (86.7%) | *<0.0001* |
| Vancomycin (10µg) | 66 (97.0%) | 2 (3.0%) | 0 (0%) | 59 (98.3%) | 1 (1.7%) | 0 (0%) | *0.92* |
| Erythromycin (15µg) | 40 (58.8%) | 2 (2.9%) | 26 (38.2%) | 11 (18.3%) | 2 (3.3%) | 47 (78.3%) | *<0.0001* |

* Fisher's exact test was used when necessary.

## Discussion

Although many studies have been carried out on the detection of enterotoxin A or *sea* gene in food, few researchers have been conducted for its detection in clinical isolates. The study of Naffa *et al*. (2006) in Jordan showed that from among 100 different clinical isolates, *sea*, *sec*, and *sea* along with *sec* were detected in 15.4%, 4% and 4% of the strains, respectively. However, none of the isolates had *sed*, *seb* and *see* (17).

In another study conducted by Koltz *et al.* (2003) in Germany, 93 isolates of stool samples were studied using Real time PCR and SET-RPLA(agglutination) and it was shown that TaqMan Real Time PCR method could detect 12 (12.9%) *sea* carrying isolates. The number of isolates carrying *seb*, *sec1*, *sed* were 9 (9.7%), 20 (21.5%) and 13 (14.0%), respectively. The rate was lower in agglutination method and the number of isolates for SEA, SEB, SEC and SED toxins were 7 (7.5%), 8 (8.6%), 19 (20.4%) and 7 (7.5%), correspondingly (18). This maybe because some strains produce lower level of enterotoxins below the sensitivity of SET-RPLA as a result of mutations in regulatory regions as described by the others (14).

In the present study, 60 isolates (46.9%) of the total 128 clinical *S. aureus* isolates contained *sea* gene. This prevalence rate was totally much more than the other studies. However, the study of Fluer *et al.* (2005) in Russia was conducted using indirect hemagglutination and it showed that the prevalence rate of the said gene in 28 blood isolates was 75.6% which is higher than that of blood isolates of current study (28.6%) (19). Moreover, they reported that the mentioned rate for strains isolated from pneumonia and infectious wound cases were 42.1% and 3%, respectively (19). Futhermore, the study of Becker *et al.* (2003) conducted on 219 *S. aureus* isolates of blood in Germany indicated that *sea* was the most frequent (17.4%)enterotoxin gene (8).

The statistical analysis of current data using Fisher's exact test indicated that the percentage of *S. aureus* carrying *sea* depends on the type of infection ($P < 0.01$). However, there was no significant relationship between the percentage of the very gene and the patients' sex and the hospital where samples came from. On the other hand, we found that presence of *sea* gene was significantly associated with higher antibiotic resistance. We are curious why those isolates carrying *sea* gene are more resistant to antibiotics. The enterotoxin producing strains may survive immune system attacks better than those isolates not producing it. In addition, mutant resistant isolates can also emerge due to continuous encounter with different antibiotics in hospitals (20). Thus, this will lead to evolution of superbug clones producing enterotoxins.

Considering the findings of the present study and comparing them to other studies, it can be stated that although the infection type was effective on the prevalence rate of the said gene in *S. aureus* isolates, the distribution of the isolates containing this gene might be higher in our society. This is due to many differences in the prevalence rate of *sea* in different studies. However, we should consider that detection of enterotoxin genes is not always concurrent with the toxin production. As we mentioned before, this maybe due to lower level of toxin productions or mutations in regulatory regions (14). Furthermore, high prevalence of antibiotic resistance in *sea* producing isolates strongly suggests that vancomycin is the only choice drug for treatment.

According to the relevant studies, the superantigenic role of enterotoxin A in the incidence of toxic shock and intensification of infections caused by *S. aureus* is clearly specified (1). A fatal case of endocarditis along with toxic shock resulting from enterotoxin A producing strain was reported in a young woman in Emirates in 2003 (21). This clearly shows the significance of enterotoxin A producing strains in stimulating inflammatory cytokines and its toxic effects on vascular endothelium layers and causes tissue damages.

Although TSST-1 and enterotoxin B were not the focus of attention in our study, the effective role of specialized antibodies is properly demonstrated in treating infections caused by TSST-1 and enterotoxin B producing strains (22, 23). Hence, the rapid detection of *sea* in clinical samples can provide the ground for preventing severe complications. In conclusion, the significant relationship between the type of infection and *S. aureus* isolates carrying *sea* indicates the interaction quality of the *S. aureus* pathogen and the host as well as the pathogenic role of *S. aureus*. In previous studies, the main emphasis was put on this gene and its expression on food samples. Therefore, the file examining the role of the very pathogen in the multi-functional pathogenesis will remain open. In addition, the expansion of antibiotic resistant *S. aureus* toxin generating clones in hospitals and the society might be preventable by taking preventive measures.

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