Prevalence of *seg*, *seh* and *sei* Genes among Clinical and Nasal *Staphylococcus aureus* Isolates in Palestine

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GA and AB managed the analyses of the study and performed the statistical analysis. Authors GA and KA wrote the first and the final draft of the manuscript. Authors GA and KA designed the study and wrote the protocol. Authors GA, NJ and YS managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

**Aims:** To investigate the presence of the staphylococcal enterotoxin genes *seg*, *seh* and *sei* among clinical and nasal isolates.

**Place and Duration of Study:** Department of Biology and Biotechnology, An-Najah N. University, Palestine, in 2011.

**Methodology:** A total 124 *S. aureus* isolates were collected, forty three were nasal and 81 were clinical isolates. PCR technique was used to detect enterotoxin genes *seg*, *seh* and *sei*, *mecA* gene and analysis of SCC*mec* types. Enterotoxigenic strains were also typed using coagulase typing kit.

**Results:** Fifty two (41.9%) isolates were positive for one or more of these enterotoxin genes. The prevalence of toxin genes among *S. aureus* isolated from nasal swabs 25/43 (58.1%) was higher than those isolated from clinical samples 27/81 (33.3%). Combination of the toxin genes was noted only in MSSA isolate from both nasal swabs and clinical samples. Distribution of toxin genes in MSSA isolates was higher (49.5%) than those in MRSA isolates (21.2%). SCC*mec* typing showed that the MRSA enterotoxigenic strain were belonged to types II, III and IVa. MRSA strains were found to belong to coagulase
serotypes II, III and VII, while MSSA strains were belonged to serotypes II-VII. In nasal samples, 16/25 (64.0%) of enterotoxigenic strains showed the genotype seg+/sei+, while in clinical samples 1/27 (3.7%), 1/27 (3.7%) and 3/27 (11.1%) of enterotoxigenic strains showed the genotypes seg+/seh+, seg+/sei+ and seg+/seh+/sei+, respectively. This study showed that the majority of the isolates 42/124 (33.9%) were seg+, while none of nasal strains harbored seh gene.

**Conclusion:** The prevalence of seg, seh and sei genes in the S. aureus isolated from nasal swabs differed significantly from those obtained from clinical samples, as well as the prevalence of the same genes in MSSA differed significantly from those in MRSA. In addition, S. aureus isolates from clinical and nasal swabs could serve as a possible reservoir of newly described seg, seh and sei genes.

**Keywords:** Enterotoxigenic S. aureus; toxin genes; seg; seh; sei.

### 1. INTRODUCTION

*Staphylococcus aureus* is considered one of the most important pathogen to humans and animals. In human, many different strains of *S. aureus* are capable of causing a wide variety of diseases ranging from skin and tissue infections, toxin-mediated diseases, pneumonia, and bacteremia [1]. *Staphylococcus aureus* infections can be acquired through both hospital and community settings. However, many of these infections are difficult to treat because of evolved resistance to antimicrobial drugs. The emergence of methicillin-resistant *S. aureus* (MRSA) strains and other antimicrobial agents has become a major concern, especially in the hospital environment, because of increased mortality due to systemic MRSA infections [2]. About 30% of individuals carry this organism in the anterior nares at any given time [3], and more than 70% of isolates recovered from healthy population were enterotoxigenic [4].

There are several types of virulence factors produced by this pathogen, which contribute in different ways to pathogenicity. These factors include degradative enzymes, surface-associated factors and superantigenic toxins [5]. Staphylococcal superantigens (SAgs) including staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) were originally identified in *S. aureus*. Several SEs were designated as SE-like (SEI) since they either lack emetic properties or their emetic activities have not been examined. More than 20 types of SEs/SEIs which are usually subdivided into classical SEs (SEA-SEE), newly described SEs (SEG-SEI and SER-SET) and SEIs (SEIU-SEIQ, SEIU and SEIV) [6,7]. These toxins are encoded by accessory mobile genetic elements, including prophages, plasmids, chromosomal pathogenicity islands (SaPIs or vSa), or by genes located next to the staphylococcal cassette chromosome (SCC) implicated in methicillin resistance [6,8,9].

Staphylococcal enterotoxins are a group of toxins resistant to heat treatment, low pH and proteolytic digestive enzymes such as pepsin, with low molecular mass proteins of approximately 27–31 kDa, similar in composition and biological activity but differ in antigenicity [6,10,11]. Products of seg, seh and sei genes showed emetic activity with an animal model [12], but it is not yet clear if seg and sei genes are responsible for food poisoning in humans. There is limited information about the prevalence of the recently identified enterotoxins in human population in comparison to the classical enterotoxins.

Enterotoxin-producing *S. aureus* plays an important role in food intoxications. Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are considered as the major source of food contamination, via manual contact or through respiratory
secretions. Staphylococcal food poisoning, caused by ingestion of enterotoxins is characterized by emesis and in some cases is accompanied by gastro-enteritis.

To the best of our knowledge, there are no published reports about the occurrence of these newly described staphylococcal enterotoxin genes in Palestinian clinical and nasal S. aureus isolates. The purpose of the present study was to investigate the presence of the SE genes seg, seh and sei in sets of MSSA and MRSA isolates collected from different clinical samples and nasal swabs from healthy students during 2011.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

A total of 124 S. aureus isolates were collected in 2011, in which 43 were nasal swab isolates and 81 were clinical isolates. Nasal swabs were obtained from 95 healthy students, Dept. of Biology and Biotechnology, An-Najah National University-Palestine, including 15 males and 80 females. Their ages varied between 19 and 24 and all declared that have not received antibiotics recently. Nasal samples were collected from students with swab rubbed inside one nostril followed by the other, yielding a paired swab sample. After sampling, swabs were immediately transferred into 5 mL nutrient broth and incubated for 18-24 h at 37ºC. Thereafter, 100 μL of the nutrient broth were sub cultured for identification. Clinical isolates were obtained from three different health centers in northern Palestine in the same time period of the study. The patients were 35 females and 46 males, between 5 and 70 years old. S. aureus was isolated from all the pathological samples (36 urine samples, 7 semen samples, 8 blood samples, 15 wound samples and 15 diabetic foot samples). Replicate isolates from the same patient were excluded. Identification of S. aureus was confirmed on the basis of Gram stain, catalase, culture properties on mannitol salt agar, detection of hemolysis on blood agar, and coagulase reaction.

2.2 Antimicrobial Susceptibility Testing

S. aureus strains were tested for Methicillin resistance using the disk diffusion method [13]. Methicillin (5 µg) discs (Oxoid) were used in this test. Inhibition zones were determined in accordance with procedures of the Clinical and Laboratory Standards Institute (formerly NCCLS) [14]. Isolates were categorized as susceptible if inhibition zones were ≥14 mm. Otherwise, the isolates were considered as resistant. All methicillin resistant S. aureus strains identified by disk diffusion method were also confirmed by the presence of gene mecA using PCR as described previously [15]. Methicillin-resistant control strains from our department collection and methicillin-susceptible reference (S. aureus ATCC 25923) were used in this study.

2.3 DNA Extraction

S. aureus genome was prepared for PCR by boiling and cooling method. Briefly, cells were scraped off an overnight nutrient agar plate with a sterile loop, washed twice in 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.5]), re suspended in 0.5 ml of sterile distilled H2O, and boiled for 10-15 min. The cells then were incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 X g for 5 min, and the supernatant containing the released DNA was transferred into a new Eppendorf tube and stored at -20ºC for used.
2.4 PCR Amplification

2.4.1 PCR amplification of mecA

The mecA gene was detected by PCR with specific primers: mecA1 (5’-TGG CTA TCG TGT CAC AAT CG-3’) and mecA2 (5’-CTG GAA CTT GTT GAG CAG AG-3’) were used for amplification of a fragment of 310 bp [15]. The PCR reaction mix with a final volume of 50 μL, was composed of 5 μL of extracted DNA, 0.4 μM of each primer, 0.2 mM of each dNTP, 1.8 mM of MgCl₂, 1X PCR buffer and 1.5 U of Taq DNA polymerase. The amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany), after initial denaturation stage at 92°C for 1 min, 40 cycles of amplification were performed as follows: denaturation at 92°C for 20 s, annealing at 58°C for 20 s, and extension at 72°C for 20 s, with increment of 2 s per cycle for the denaturation and extension segments. At the end of the cycles, the reaction mixture was achieved with final extension at 72°C for 5 min. After amplification PCR products were analyzed by electrophoresis on 2% (w/v) agarose gel. Methicillin-resistant control strains from our department collection were used in this study.

2.4.2 Multiplex PCR assay for SCCmec typing

The primer sets used for SCCmec typing were described previously [16]. Each PCR reaction mix (50 μL) contained 1X PCR buffer, 1.5 U Taq DNA polymerase, 3 mM MgCl₂, 5 μL primer mix (2 mM in TE buffer for each primer), 0.2 mM dNTPs and 3 μL DNA template. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 5 min at 94°C followed by 30 cycles of initial denaturation 94°C for 30 s, 57°C for 1.5 min and 72°C for 1.5 min ending with a final extension step at 72°C for 5 min, and followed by a holding step at 4°C. The PCR products (10 μL) were analyzed by electrophoresis on 2% agarose gel. The SCCmec type was determined on the basis of the band pattern obtained. Each individual PCR yielded the fragments of expected size: i.e. 613, 287, 243, 776, 1000, 677, 1242, 663 and 325 bp for subtypes I, II, III, IVa, IVb, IVc, IVd, IVh and V, respectively. Control strains for different SCCmec types from our department collection were used in this study.

2.4.3 Detection of the toxin genes

The primer sets used for the detection of seg, seh and sei genes were described previously [17]. Each PCR reaction mix (50 μL) contained 1X PCR buffer, 1.5 U Taq DNA polymerase, 2 mM MgCl₂, 0.4 μM of each primer, 0.2 mM dNTPs and 3 μL DNA template. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 1 min at 94°C followed by 30 cycles at 94°C for 30 s for denaturation, and extension at 72°C for 30 s. Different annealing temperatures were used 55°C, 46.4°C and 50°C for 30 s for seg, seh and sei genes, respectively. Final extension was carried out at 72°C for 5 minutes. After amplification PCR products were analyzed by electrophoresis on 2% agarose gel. Amplification of non template controls was attempted with each reaction to determine if DNA contamination occurred. The mixture of genomic DNA from S. aureus reference strains (ATCC 51811 for seh and S. aureus ATCC 25923 for seg and sei genes) were used for PCR of each set as a positive control.
2.5 Coagulase Typing

Coagulase serotyping was performed with the coagulase Seiken typing kit (Denka-Seiken, Japan) according to the manufacturer's instructions.

2.6 Statistical Analysis

A $\chi^2$ test was used to study the correlation between the prevalence of the enterotoxin genes in different groups of S. aureus isolates. Statistical analysis was done using statistical package, SPSS windows version 15. A value of $P<0.05$ was considered statistically significant.

3. RESULTS

Our results showed that all the MRSA isolates detected by disk diffusion method were displayed positive amplifications of the meca gene. Detection of enterotoxin genes by PCR showed that fifty two (41.9%) of S. aureus isolates were positive for one or more of these genes. The prevalence of toxin genes among S. aureus isolated from nasal swabs 25/43 (58.1%) was higher than those isolated from clinical samples 27/81 (33.3%). Combination of the toxin genes was noted only in MSSA isolates from both nasal swabs and clinical samples. The prevalence of toxin genes in MSSA isolates was higher (49.5%) than those in MRSA isolates (21.2%). In nasal swab samples, 16/25 (64.0%) of enterotoxigenic strains showed the genotype seg*/sei*, while in clinical samples 1/27 (3.7%), 1/27 (3.7%) and 3/27 (11.1%) of enterotoxigenic strains were seg*/seh*, seg*/sei* and seg*/seh*/sei*, respectively. This study showed that the majority of the isolates 42/124 (33.9%) were seg*, while 10/124 (8.1%) were seh*. No seh gene was identified among the nasal swabs isolates. Prevalence of seg, seh and sei genes in MRSA and MSSA isolated from clinical samples and healthy nasal swabs are presented in Table 1. The $X^2$ test showed that the prevalence of seg, seh and sei genes in the S. aureus isolated from nasal swabs differed significantly from those obtained from clinical samples and the $P$ value was 0.008, while the prevalence of the same genes in MSSA differed significantly from those in MRSA with a $P$ value was 0.005.

Table 1. Prevalence of seg, seh and sei genes in MRSA and MSSA isolated from clinical and healthy nasal samples

| Source of S. aureus isolates | Prevalence of toxin genes | No. and % of enterotoxigenic culture |
|-----------------------------|---------------------------|--------------------------------------|
| Nasal swab (n=43)$^a$      | seg: 24 (55.8%), seh: 0 (0.0%), sei: 17 (39.5%) | 25 (58.1%) |
| MRSA (Nasal swab) (n=4)    | 1 (25%)                   | 1 (25.0%) |
| MSSA (Nasal swab) (n=39)   | 23 (59.0%)                 | 24 (61.5%) |
| Clinical isolates (n=81)$^b$| seg: 18 (22.2%), seh: 10 (12.3%), sei: 7 (8.6%) | 27 (33.3%) |
| MRSA (Clinical) (n=29)     | 2 (6.9%)                   | 6 (20.7%) |
| MSSA (Clinical) (n=52)     | 16 (30.8%)                 | 21 (40.4%) |
| Total isolates (124)       | seg: 42 (33.9%), seh: 10 (8.1%), sei: 24 (19.4%) | 52 (41.9%) |
| Total MRSA isolates (n=33)$^b$| seg: 3 (9.1%), seh: 4 (12.1%), sei: 0 (0.0%) | 7 (21.2%) |
| Total MSSA isolates (n=91)$^b$| seg: 39 (42.9%), seh: 6 (6.6%), sei: 24 (26.4%) | 45 (49.5%) |

$^a$ Statistically significant association after analysis ($P < 0.05$), the $P$ value was 0.008.

$^b$ Statistically significant association after analysis ($P < 0.05$), the $P$ value was 0.005.
The $X^2$ test also showed that the overall presence of the enterotoxin genes was independent of the coagulase serotypes. The SCCmec typing showed that the MRSA enterotoxigenic strains isolated from both clinical and nasal swab samples were belonged to type II, III and IVa. Coagulase typing showed that all the MRSA strains were found to belong to coagulase serotypes II, III and VII, while MSSA strains were belonged to serotypes II-VII and 2 strains were not detected. Coagulase type, SCCmec type of enterotoxigenic S. aureus isolated from nasal and different clinical sources are presented in Table 2.

**Table 2.** Coagulase type, SCCmec type of enterotoxigenic S. aureus isolated from nasal and different clinical sources in Palestine

| Source of sample | Toxingene | Sample no. | SCCmec type | Coagulase type |
|------------------|------------|------------|-------------|---------------|
| Nasal (MRSA)     | seg*       | 1          | IVA         | II            |
| Clinical (MRSA)  | seh*       | 1          | IVA         | II            |
| Diabetic ulcer   | seh*       | 1          | IIA         | III           |
| Blood            | seg*       | 1          | IVA         | II            |
|                  | seh*       | 1          | IVA         | II            |
| Semen            | seh*       | 1          | IIA         | III           |
| Wound            | seh*       | 1          | IIA         | III           |
| Nasal (MSSA)     | Seg*/seI*  | 1          | II          | ND*           |
|                  | seg*       | 1          | III         | ND*           |
|                  | seg*/seI*  | 4          | III         | ND*           |
|                  | seh*       | 1          | III         | ND*           |
|                  | seg*       | 1          | III         | ND*           |
|                  | seh*       | 1          | III         | ND*           |
|                  | seh*/seI*  | 1          | III         | ND*           |
| Clinical (MSSA)  | seg*       | 3          | III         | ND*           |
| Urine            | seh*       | 1          | III         | ND*           |
|                  | seh*       | 1          | III         | ND*           |
|                  | seh*/seI*  | 1          | III         | ND*           |
|                  | seh*       | 1          | III         | ND*           |
| Semen            | seh*/seI*  | 1          | II          | ND*           |
| Blood            | seh*/seI*  | 1          | IV          | ND*           |
|                  | seh*       | 1          | VI          | ND*           |
|                  | seh*/seI*  | 1          | II          | ND*           |
| Wound            | seh*       | 1          | III         | ND*           |
|                  | seh*/seI*  | 2          | II          | ND*           |
| Diabetic ulcer   | seh*/seI*  | 1          | III         | ND*           |
|                  | seh*       | 1          | III         | ND*           |

* Not detected
4. DISCUSSION

Staphylococcal super antigens are virulence factors associated with S. aureus, which have pivotal roles on colonization, modulating the host immune response and also ability to cause food poisoning in humans [5,7,18]. Results from different reports have shown the high incidence of genes encoding new SEs and SEIs among food-borne S. aureus [6]. Of these new enterotoxins, only seh+ strains have clearly been involved in staphylococcal food poisoning outbreaks. Our results showed that all S. aureus isolated from nasal swabs did not carry she, meaning that these strains had not ability to cause food poisoning in humans. However, food poisoning outbreaks due to seg+, seh+ and sei+ S. aureus without producing any of the classical SEs have been described previously [4,8,19,20]. It was pointed out that, the presence of seg and sei genes did not mean the production of enough quantities of SEG and SEI to cause the food poisoning [4]. It is possible that the strains harboring new enterotoxin genes specially seg and sei may coexist with bacterial pathogens other than S. aureus that made tracing the origins of food poisoning cases more complicated [20], or the presence of multiple SE genes in S. aureus makes it less clear whether any single toxin is responsible for staphylococcal food poisoning [18]. It was shown that new enterotoxin genes mainly seg, sei, sek and seg were found in isolates from patients rather than strains from food samples and food poisoning [21]. Accordingly, these superantigens may contribute to enhance the persistence of infection. However, as their role as virulence factors in infection is still purely speculative, the main interest of the enterotoxins resides in their ability to cause food poisoning. Clinical S. aureus strains involved in staphylococcal toxic shock syndrome and scarlet fever without producing classical SE’s or toxic shock syndrome toxin 1, leading to the suggestion that SEG or SEI toxins caused these diseases [22].

In Palestine, the prevalence of classical staphylococcal enterotoxin genes was determined from human clinical samples and it has ranged 41.2% to 63.3% [23,24]. In this study, up to 41.9% of S. aureus strains isolated from nasal swabs of healthy students and different clinical isolates carried seg, seh and/or sei genes. The prevalence of enterotoxigenic S. aureus isolates carried one or more of seg, seh and/or sei genes from different reports has ranged from 30% to 68.6 % [1,4,5,12,17,25-30]. The prevalence of seg, seh and sei genes in this study was 33.9%, 8.1% and 19.4%, respectively. The prevalence of seg gene in previous reports has ranged from 8.6% to 63.9%, seh from 0% to 54% and sei from 11.5% to 62.8% [1,4,5,12,17,20,25-30]. This variation in the prevalence of these genes between these studies might be attributable to different factors such as the geographical difference, which may be further affected by the different ecological origins of the isolated strains (food, humans and animals), type of sample (blood, urine, nasal swab, milk, meat), number of samples and methodology.

Our data showed that 20/124 (16.9%) of isolates carried the genotype seg+/sei+. The prevalence of strains had seg+/sei+ genotype ranged from 0.7% to 62.4% [1,4,5,12,17,20,25-30]. The majority of sei and seg distribution studies showed that these genes were always detected together in S. aureus [22,26,29,31,32]. The high rate of coexistence of seg and sei in S. aureus isolates suggests that these genes may exist as members of egc in most seg+/sei+ isolates. It is possible that sei is absent in some seg-positive S. aureus strains [4,20,27,33] or may also be explained by mispriming due to the choice of polymorphic regions as the primer target, which makes its detection impossible [34].

This study showed that seg gene was the most commonly detected among positive enterotoxigenic strains, then sei gene, while seh gene was the lowest. These results were in
contrast to previous study [4], which reported that seh gene was the most frequently detected among positive enteroxigenic strains isolated from humans with food poisoning and from healthy humans. Our results were consistent with a previous report [27], which showed that seg gene was the most frequently detected among clinical strains, then sei gene, while seh gene was the lowest. Other previous reports showed that the distribution of seg and sei genes from different clinical isolates had the same prevalence or approximately equal, while seh gene detected in very low or absent [26,28,32,35-37].

Our results showed that seg and sei genes were detected more frequently in MSSA. These results were inconsistent with the previous reports which showed that seg and sei genes were detected more frequently in MRSA isolates [28,32]. Recent report showed no correlation between number of SE genes and degree of antimicrobial resistance [37]. MRSA is produced when MSSA acquires a mobile genetic element, SCCmec. Toxin-producing MSSA may also change the pathogenicity of established MRSA by the transfer of virulence factors via mobile genetic elements or plasmids.

Coagulase serotyping revealed that the 45 enterotoxigenic S. aureus isolates were serologically diverse and comprised a heterogeneous population with 6 coagulase serotypes. Although the identified S. aureus coagulase serotypes harbored the tested SE genes either singly or in combination, the X2 test showed that the overall presence of the enterotoxin genes was independent of the coagulase serotypes. Horizontal transfer of these genes could occur among isolates of different coagulase serotypes.

5. CONCLUSION

Our findings in this pilot study support the conjecture that S. aureus isolates collected from both clinical and nasal swabs could serve as a possible reservoir of newly described seg, seh and sei genes. The prevalence of seg, seh and sei genes in the S. aureus isolated from nasal swabs differed significantly from those obtained from clinical samples, as well as the prevalence of the same genes in MSSA differed significantly from those in MRSA. Further studies are needed to confirm the expression of these new enterotoxin genes by clinical strains of S. aureus, and to assess their significance in diseases. Based on our knowledge, the present study represents the first investigation carried out in Palestine regarding the relationship among the origin (nasal swabs or clinical strains), enteroxigenicity and Methicilline resistant or sensitive S. aureus isolates.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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