

Toxicogenicity of *Staphylococcus aureus* isolates from Northern Palestine

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Abstract - A total of 68 *Staphylococcus aureus* strains isolated from different human clinical samples in the North of Palestine were examined to detect staphylococcal enterotoxin (SE) genes A (*sea*), B (*seb*), C (*sec*), D (*sed*) and (*see*). Of the total isolates examined, 41.2% (28/68) were enterotoxigenic *S. aureus*. Twelve strains (42.9%) of enterotoxigenic *S. aureus* harbored *sea*-gene, ten strains (35.7%) were carried *see*-gene, six strains (21.4%) were positive for *sec*-gene. None of these enterotoxigenic *S. aureus* isolates harbored more than one of toxin genes. The presence of these toxin genes and other genes not be detected here might play a role in process of pathogenesis of *S. aureus* disease other than food poisoning but this cannot be substantiated by the results of the present study.

Keywords: Enterotoxigenic *S. aureus*, staphylococcal enterotoxins, SEs, Palestine.

It is noteworthy that *S. aureus* is an important pathogen due to combination of antibiotic resistance, invasiveness and toxin-mediated virulence. This pathogen plays a significant role in nosocomial infections as well as community acquired-diseases.¹ Most strains secrete a group of enzymes and cytotoxins which includes hemolysins, nucleases, proteases, lipases, hyaluronidase, and collagenase. The main function of these proteins may be to convert local host tissues into nutrients required for bacterial growth. Some strains of this pathogen produce a wide variety of toxic proteins including enterotoxins (SEs), toxic shock syndrome toxin-1 (TSST-1), exfoliative toxins (ETs) and leukocidin.² These toxins are responsible for specific acute staphylococcal toxemia syndromes such as scalded skin syndrome and staphylococcal food poisoning. The existence of SE genes in *S. aureus* isolates is necessary for these strains to cause food poisoning or potential factors which contribute in other diseases.³⁻¹³

To date staphylococcal enterotoxins (SEs) have been classified into 14 different types, which share structural and sequence similarities.¹ These enterotoxins are heat-stable and resistant to the action of digestive enzymes. The most common types of these enterotoxins are SEA to SEE. Toxicogenicity of *Staphylococcal aureus* isolates from human clinical samples have been previously studied using different methods.^{7,12,14-16} This study was conducted to investigate the incidence of enterotoxin genes A (*sea*), B (*seb*), C (*sec*), D (*sed*) and (*see*) in *S. aureus* isolates recovered from different human clinical samples in the North of Palestine as this has not been investigated previously.

Materials and Methods

A total of 68 isolates of *S. aureus* were recovered from different human clinical samples in the North of Palestine. These samples were urine (n=46), semen (n=18) and diabetic foot swab (n=4) collected from hospitals and private medical laboratories between May and July of 2005. In our laboratory in order to confirm the diagnosis of these samples, they were cultured on 5% sheep blood agar, nutrient agar and subsequently on mannitol salt agar. Gram stain and culture characteristics (colony morphology, pigmentation, and hemolysis) were used for presumptive identification of all isolates. Colonies suspected as staphylococcus were examined to see if they were *S. aureus* by the coagulase test tube method. All coagulase-positive isolates were further tested using the API STAPH-IDENT, 32 Staph (bioMerieux SA, 69280 Marcy-l'Etoile, France) to confirm their identification. Total DNA was isolated from about 10 colonies of the bacteria as described previously with minor modifications.¹⁷ Bacterial cells were washed once with 1.0 ml of 0.02 M sodium phosphate (Na₂HPO₄·2H₂O) pH 7.4 in 0.9% NaCl and centrifugation at 12000Xg for 10 min. The pellet was resuspended in 200 µl of lysis buffere (1mM EDTA, 10 mM Tris-chloride, pH 8) with 12 U lysostaphin (Sigma) and incubated for 45-60 min at 37°C. Then 4.5 U of proteinase K (MO BIO) were added and incubated for 45 min at 60°C, then for 10 min at 95°C. The total DNA is spinned at 12,000 X g for 15 s. and kept at -20°C for DNA amplification.

The *sea*, *seb*, *sec*, *sed* and *see* gene sequences were detected using the primer pairs described previously.^{10,18}

Table 1 - Toxin gene profiles of 28 enterotoxigenic *S. aureus* isolates recovered from human clinical samples in the North of Palestine

Source of clinical sample	No. of clinical samples	No. (%) of toxin genes	Total no. (%) of enterotoxi genic samples
Urine	46	SEA= 8 (17.4%) SEE= 6 (13%) SEC= 2 (4.3%)	16/28 (57.1%) 16/28 (57.1%)
Semen	18	SEA= 4 (22.2%) SEE=2 (11.1%) SEC=4 (22.2%)	10/28 (35.7%)
Diabetic foot swab	4	SEE= 2 (50%)	2/28 (7.2%)
Total	68	28 (41.2%)	

For PCR amplification, the reaction mixture (30 µl) was performed as previously described.¹⁷ It included 10 pmol of each primer, 0.2 mM of each deoxynucleoside triphosphate (PeQLab), 1 X PCR reaction buffer (PeQLab), 1.5 mM of MgCl₂ (PeQLab), 1U of *Taq* DNA polymerase (PeQLab). Finally, 1 µl of DNA template was added to each 0.2-ml reaction tube and the total volume was adjusted by adding sterile double distilled water. The amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf) with the following program: 1 X 4 min precycle at 94°C followed by 30 PCR cycles (2 min at 94°C for denaturation, 2 min at 55°C for annealing, and 1 min at 72°C for extension). At the end of the cycles, the reaction mixture was maintained at 72°C for 5 min. PCR products were separated by electrophoresis in 2% agarose gel and stained with ethidium bromide. All primers used in this study were synthesized by Integrated DNA Technologies (IDT), Inc. USA.

Results

Enterotoxigenic *S. aureus* isolates were identified in 28 (41.2%) of 68 *S. aureus* isolates recovered from different human clinical samples in the North of Palestine. The majority of these enterotoxigenic isolates 12 (42.9%) harbored the sea gene. None of these enterotoxigenic *S. aureus* isolates harbored more than one toxin gene. Overall, 40.6% (26/64) of the urogenital strains produced enterotoxins. The toxin gene profiles of enterotoxigenic *S. aureus* isolates are presented in Table (1).

Discussion

In this study, three toxin genes sea, sec and see were detected. The majority of enterotoxigenic *S. aureus* isolates carried toxin gene sea. This result was consistent with some previous studies in which most enterotoxigenic *S. aureus* isolates usually carried toxin gene

sea.^{5,12,14,16,19-24} However, our result was in contrast to other studies where most enterotoxigenic *S. aureus* isolates usually carried toxin gene seb, sec, sed or see.^{3,6-7,15,25-27}

The prevalence of enterotoxigenic *S. aureus* in the human clinical samples we obtained from North Palestine was 41.2%. If other toxin genes were detected and increased the number of isolates the prevalence obtained might be changed. These findings do not suggest a possible role for enterotoxins in the pathogenesis of urogenital disease; this might be due to small number of test samples and need to detect other toxin genes. The prevalence of enterotoxigenic *S. aureus* from different human clinical samples has been reported previously and has ranged from 17.8% to 86.6%.^{4,6,12,21-23,27} The prevalence of staphylococcal enterotoxin producing strains from human clinical samples differs among studies in different countries or in different areas of the same country. This might be due to differences in ecological origin of strains, the sensitivity of detection methods, detected genes and number of samples and the type of clinical samples included in these studies. This study showed that no enterotoxigenic isolates carried more than one gene. This result was in contrast to other studies which have showed that some enterotoxigenic isolates had more than one gene.^{3-5,13-16,20-23,26}

Although the number of *S. aureus* isolates tested in this study was fairly small, it is a representative sample giving a picture of the general situation in Palestine. Further studies are needed to find the relationship between these genes and pathogenicity of this pathogen.

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