Toxigenicity 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 enterotoxins (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 se-gene, ten strains (14.7%) 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 these genes. The presence of these two genes and other genes not been 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 cyto toxins 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 proteins 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.2,4,9

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. Toxigenicity of Staphylococcus aureus isolates from human clinical samples have been previously studied using different methods.12,13,14 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 (color 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.5 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 12000 rpm for 10 min. The pellet was resuspended in 200 mL of lysis buffer (10mM EDTA, 10 mM Tris-chloride, pH 8) with 12 U lysozyme (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 was spinned at 12,000 rpm 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.15,18
Table 1 - Toxin gene profiles of 28 enterotoxigenic S. aureus isolates recovered from human clinical samples in the North of Palestine

<table>
<thead>
<tr>
<th>Source of clinical sample</th>
<th>No. of clinical samples</th>
<th>No. (%) of toxin genes</th>
<th>Total no. (%) of enterotoxin genic samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>46</td>
<td>SEA = 8 (17.4%)</td>
<td>16/28 (57.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEE = 6 (13.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEC = 2 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Semen</td>
<td>18</td>
<td>SEA = 4 (22.2%)</td>
<td>10/28 (35.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEE = 2 (11.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEC = 4 (22.2%)</td>
<td></td>
</tr>
<tr>
<td>Diabetic foot swab</td>
<td>4</td>
<td>SEE = 2 (50%)</td>
<td>2/28 (7.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td></td>
<td>28 (41.2%)</td>
</tr>
</tbody>
</table>

For PCR amplification, the reaction mixture (30 µl) was performed as previously described. 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 (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 usually carried toxin gene sea. However, our result was in contrast to other studies where most enterotoxigenic S. aureus isolates usually carried toxin gene seb, sec, sed or see. The prevalence of enterotoxigenic S. aureus in the human clinical samples we obtained from Northern 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%. 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.

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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References


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