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Original Article

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 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. Toxigenicity 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 inorder 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. aures 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%)	16/28 (57.1%)
		SEE= 6 (13%)	
		SEC= 2 (4.3%)	16/28 (57.1%)
Semen	18	SEA= 4 (22.2%)	10/28 (35.7%)
		SEE=2 (11.1%)	
		SEC=4 (22.2%)	
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 starins 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 pathogenecity of this pathogen.

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References

1. Loir YL, Baron F and Gautier M: *Staphylococcus aureus* and food poisoning. *Genet Mol Res,* 2003; **2:** 63-76.

2. Dinges MM, Orwin PM and Schlievert PM: Exotoxins of *Staphylococcus aureus. Clin Microbiol Rev,* 2000; 13: 16-34.

3. Adesiyun AA, Lenz W and Schaal KP: Phage susceptibility, enterotoxigenicity and antibiograms of Staphylococcus aureus strains isolated from human wounds and diarrhoea. *Zentralbl Bakteriol,* 1992; **277:** 250-259

4. Adesiyun AA, Prabhakar P, Ali C, *et al:* Characteristics of Staphylococcus aureus strains isolated from clinical and non-clinical human sources in Trinidad: susceptibility to bacteriophages and antimicrobial agents, and toxigenicity. *Zentralbl Bakteriol,* 1995; **282:** 519-532.

5. Lehn N, Schaller E, Wagner H, *et al:* Frequency of toxic shock syndrome toxin- and enterotoxin-producing clinical isolates of Staphylococcus aureus. *Eur J Clin Microbiol Infect Dis,* 1995; **14:** 43-46.

6. Roder BL, Eriksen NH, Nielsen LP, *et al:* No difference in enterotoxin production among *Staphylococcus aureus* strains isolated from blood compared with strains isolated from healthy carriers. *J Med Microbiol,* 1995; **42:** 43-47.

7. Tortora JC, De Sousa TL, Lourenco MC, *et al:* Nosocomial occurrence of enterotoxigenic multiresistant Staphylococcus strains in Rio de Janeiro. *Rev Latinoam Microbiol,* 1996; **38:** 1-6.

8. Jarraud S, Cozon G, Vandenesch F, *et al:* Involvement of enterotoxin G and I in staphylococcal toxic shock syndrome and staphylococcal scarlet fever. *J Clin Microbiol,* 1999; **37:** 2446-2449.

9. Jarraud, S, Peyrat MA, Lim A, *et al: egc*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus. J Immunol,* 2001; **166:** 669-677.

10. Stephan, R, Annemuller C, Hassan AA, *et al:* Characterization of enterotoxigenic Staphylococcus aureus strains isolated from bovine mastitis in north-east Switzerland. *Vet Microbiol,* 2001; **78:** 373-382.

11. Omoe K, Ishikawa M, Shimoda Y, *et al:* Detection of *seg, seh,* and *sei* genes in *Staphylococcus aureus* Isolates and Determination of the Enterotoxin Productivities of *S. aureus* Isolates Harboring *seg, seh,* or *sei* Genes. *J Clin Microbiol,* 2002; **40:** 857-862.

12. Efuntoye MO and Adetosoye AI: Enterotoxigenicity and drug sensitivity of staphylococci from children aged five years and below with sporadic diarrhea. *East Afr Med J,* 2003; **80:** 656-659.

13. Da Silva ER, Do Carmo LS and Da Silva N: Detection of the enterotoxins A, B, and C genes in *Staphylococcus aureus* from goat and bovine mastitis in Brazilian dairy herds. *Veterinary Microbiol,* 2005; **106:** 103-107.

14. Pickenhahn P, Hahn IF, Lenz W, *et al:* Correlation between enterotoxigenicity, tested by different ELISA-techniques, antibiotic resistance patterns and phage groups of Staphylococcus aureus strains. *Zentralbl Bakteriol Mikrobiol Hyg A,* 1987; **266:** 127-136.

15. Fueyo JM, Martin MC, Gonzalez-Hevia MA, *et al:* Enterotoxin production and DNA fingerprinting in *Staphylococcus aureus* isolated from human and food samples. Relations between genetic types and enterotoxins. *Int J Food Microbiol,* 2001; **67:** 139-145.

16. Simkovicova M, Hanzen J, Milosovic P, *et al:* Evidence of enterotoxins and toxic shock syndrome toxin (TSST-1) in strains of *Staphylococcus aureus* isolated from various biological materials. *Epidemiol Mikrobiol Imunol,* 2002; **5:** 102-106.

17. Adwan G., Abu-shanab B and Adwan K: Enterotoxigenic *Staphylococcus aureus* in raw milk in the North of Palestine. *Turk J Biol,* 2005; **29:** 229-232.

18. Akineden O, Annemuller C, Hassan AA, *et al:* Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. *Clin Diagn Lab Immunol,* 2001; **8:** 959-964.

19. Melconian AK, Brun Y and Fleurette J: Enterotoxin production, phage typing and serotyping of *Staphylococcus aureus* strains isolated from clinical materials and food. *J Hyg Lond,* 1983; **91:** 235-242.

20. Mauff G, Rohrig I, Ernzer U, *et al:* Enterotoxigenicity of Staphylococcus aureus strains from clinical isolates. *Eur J Clin Microbiol,* 1983; **2:** 321-326.

21. Adekeye JD and Adesiyun AA: Frequency of isolation of enterotoxigenic staphylococci from milk of nursing mothers in Kaduna, Nigeria. *J Hyg Lond,* 1984; **93:** 531-538.

22. Rivas M, Cordal de Bobbi ME, M.S. Raffo Palma, *et al:* Enterotoxigenicity and biochemical features of strains of *Staphylococcus aureus* of different origins. *Rev Argent Microbiol,* 1985; **17:** 69-73.

23. Al Bustan MA, Udo EE and Chugh DT: Nasal carriage of enterotoxin-producing Staphylococcus aureus among restaurant workers in Kuwait City. *Epidemiol Infect,* 1996; **116:** 319-322.

24. Gravet A, Rondeau M, Harf-Monteil C, *et al:* Predominant *Staphylococcus aureus* Isolated from Antibiotic-Associated Diarrhea Is Clinically Relevant and Produces Enterotoxin A and the Bicomponent Toxin LukE-LukD. *J Clin Microbiol,* 1999; **37:** 4012-4019.

25. De Nooij M.P, van Leeuwen WJ and Notermans S: Enterotoxin production by strains of *Staphylococcus aureus* isolated from clinical and non-clinical specimens with special reference to enterotoxin F and toxic shock syndrome. *J Hyg Lond,* 1982; **89:** 499-505.

26. Reali D: Enterotoxin A and B production in strains of *Staphylococcus aureus* isolated from human beings and foods. *J Hyg Lond,* 1982; **88:** 103-106.

27. El-Ghodban A, Ghenghesh KS, Marialigeti K, *et al:* Enterotoxins and phage typing of *Staphylococcus aureus* isolated from clinical material and food in Libya. *Arch Inst Pasteur Tunis,* 1999; **76:** 23-25.

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