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ENTEROTOXIGENECITY OF *S. AUREUS* ISOLATES RECOVERED FROM CHRONIC UROGENITAL TRACT INFECTION IN NORTH PALESTINE

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ABSTRACT

Objectives: *Staphylococcus aureus* is an important pathogen associated with diseases in a variety of hosts including humans. It produces several toxins and virulence factors that contribute to its pathogenic potential such as staphylococcal enterotoxins (SEs). This study was conducted to determine enterotoxigenicity of *S. aureus* associated with chronic urogenital tract infection by detecting *enterotoxin* genes.

Setting: This study was done in The Microbiology laboratory, An-Najah N. University, Palestine.

Methodology: A total of 90 *S. aureus* isolates recovered from clinical samples from patients suffering from chronic urogenital tract infection in the North of Palestine were used to detect the presence of staphylococcal enterotoxin genes *sea*, *seb*, *sec*, *sed* and *see* by polymerase chain reaction (PCR) assay.

Results: Out of 90 *S. aureus* isolates tested, it was found that 57 (63.3%) of these isolates harboured one or more enterotoxin genes. Up to 78.9% of the enterotoxigenic isolates possessed one SE gene. The majority of these enterotoxigenic strains (61.4%) isolated from both semen and urine samples harbored *sec* gene either alone or in combination with other genes. Also the prevalence of genes in combination was significantly more common in *S. aureus* isolates derived from urine 9/33 (27.3%), as compared to those derived from semen 3/24 (12.5%).

Conclusions: The role of enterotoxin genes in the pathogenesis of urogenital tract infection is still unknown. However, it is evident that urogenital infection can be caused by *S. aureus* strains which lack these genes. Other newly detected genes may play a role in pathogenesis.

KEYWORD: Enterotoxigenic *S. aureus*, Staphylococcal enterotoxins, SEs, Palestine.

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INTRODUCTION

S. aureus is a commensal bacterium found on host skin and respiratory tract mucosal surfaces. However, it is also associated with diseases in variety of hosts including humans. It is a significant pathogen involved in nosocomial and community-acquired infections in humans. The increasing prevalence of multidrug-resistant *S. aureus* worldwide is an additional problems and resistance to antimicrobial compounds reduces their effectiveness and increases morbidity, mortality and health care costs worldwide.[1] A variety of toxins and virulence factors produced by certain clinical isolates such as hemolysins, exfoliative toxin, and pyrogenic toxin superantigens including

enterotoxins and toxic shock syndrome toxin. These toxins are involved in the pathogenesis of staphylococcal diseases such as food poisoning, scalded skin syndrome, and toxic shock syndrome.[2] Traditionally, five classic antigenic SE types were recognised SEA to SEE encoded by *sea* to *see* genes. During the 1990s, new SE's SEG to SEJ were reported and their genes described. Recently, data resulting from partial or complete genome sequence analyses have led to a rapid description of further new genes *sek, ser* and *seu*.[3] The role of several recently described SEs as causative agents in toxin-mediated staphylococcal diseases or in enhancing the severity of *S. aureus* infections has not been confirmed.[4]

Previously, we have reported the prevalence of enterotoxigenic staphylococci from different clinical human isolates from Northern Palestine, as 41.2%. The majority of these enterotoxigenic isolates carried *sea* gene (42.9%), and none of positive isolates harboured more than one toxin gene.[5] Also limited information is available on enterotoxigenic *S. aureus* isolated from urogenital tract infection. Hence this study was conducted to determine the prevalence of enterotoxigenic *S. aureus* isolates associated with chronic urogenital tract infection by detecting *sea*, *seb*, *sec*, *sed* and *see* genes.

MATERIALS AND METHODS

During a one-year-period between August of 2005 and July of 2006, a total of 90 clinical isolates of *S. aureus* (51 derived from urine samples and 39 from semen) were recovered from patients suffering from chronic urogenital tract infection. These isolates tested to detect the presence of SE genes *sea*, *seb*, *sec*, *sed* and *see* by PCR assay. All isolates were identified in the microbiology laboratories of An-Najah National University, Palestine, by Gram stain, culture properties on nutrient agar and mannitol salt agar, detection of hemolysis on 5% sheep blood agar and by tube coagulase reaction.

Methicillin resistance was carried out using the disk diffusion method.[6] Methicillin (5µg) disks (Oxoid) were used, inhibition zones were determined in accordance with procedures of the National Committee for Clinical Laboratory Standard.[7] Isolates giving an inhibition zone e"14mm were confirmed as methicillin-sensitive isolate, while others were considered as resistant.

DNA template was isolated from approximately 10 colonies of the bacteria as described previously.[5] Bacterial cells were washed once with 1.0ml of 0.02 M sodium phosphate (Na2HPO4.2H2O) pH 7.4 in 0.9% NaCl and centrifugation at 12000Xg for ten minutes. The pellet was then resuspended in 200ìl of lysis buffer (1mM EDTA, 10mM Tris-chloride, pH 8) with 12U lysostaphin (Sigma) and incubated for 60 minutes at 37°C. Then 4.5 U of proteinase K (MO BIO) were added and incubated for 45 minutes at 60°C, and not for 10 minutes at 95°C. The total DNA is spun at 12,000 X g for 15 seconds and stored at -20°C for DNA amplification. The *sea*, *seb*, *sec*, *sed* and *see* gene sequences were detected using the primer pairs described previously.[8]

For PCR amplification, the total reaction mixture was 30ìl, and performed as previously described.[5,9] It included 10pmol of each primer, 0.2mM of each deoxynucleoside triphosphate (PeQLab), 1 X PCR reaction buffer (PeQLab), 1.5mM of MgCl2 (PeQLab), 1U of *Taq* DNA polymerase (PeQLab). Finally, 1ìl of DNA template was added to each 0.2-ml reaction tube. The amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf) with the following program: 1 X four minutes precycle at 94°C followed by 30 PCR cycles (2 minutes at 94°C, 2 minutes at 55°C, and one minute at 72°C). At the end of the cycles, the reaction mixture was maintained at 72°C for five minutes. PCR products were separated by electrophoresis in 2% agarose gel and stained with ethidium bromide. Positive reference strains for these genes were also included. All primers used in this study were synthesized by Integrated DNA Technologies (IDT), Inc. USA.

Table-I: Profile of methicilline susceptibility, enterotoxigenicity of 90 *S. aureus* isolates recovered from urogenital tract infected patients.

	Source of *S. aureus* isolates			
	Urine (51 isolates)		*Semen (39 isolates)*	
Methecillin susceptibility	*Enterrotoxigenic isolates (%)*	*Non enterotoxigenic isolates (%)*	*Enterrotoxigenic isolates (%)*	*Non enterotoxigenic isolates (%)*
Methecillin resistant	31 (60.8 %)	15 (29.4%)	21 (53.8%)	15 (38.5%)
Methecillin sensitive	2 (3.9%)	3 (5.9%)	3 (7.7%)	0 (0.0%)

RESULTS

Ninety isolates of clinical *S. aureus* from chronic urogenital tract infection were studied for methicillin resistance. It was found that fifty two (63.4%) and five out of eight methicillin sensitive isolates (62.5%) of methicillin resistant and methicillin sensitive isolates, respectively, were enterotoxigenic (Table-I).

Out of 90 *S. aureus* isolates tested for SE genes, it was found that 57 (63.3%) of these detected isolates harboured one or more enterotoxin genes. Up to 78.9% of the enterotoxigenic strains carried one SE gene. The majority of these positive toxin gene isolates 35/57 (61.4%), were *sec*-positive either single or combined with other genes.

Toxin gene profiles of these 90 enterotoxigenic *S. aureus* isolates are presented in Table-II. It also found that gene combination was significantly more common in *S. aureus* isolates derived from urine 9/33 (27.3%), while in *S. aureus* isolates derived from semen 3/24 (12.5%).

DISCUSSION

The literature shows variable results concerning the occurrence of enterotoxigenic S. *aureus* strains in clinical samples. This is probably due to the differences among the origin of clinical samples, number of test samples, sensitivity of methods, and types of enterotoxins or enterotoxin genes that were detected. Indeed prevalence of enterotoxigenic *S. aureus* observed in different human clinical samples varies markedly between reports.[5,10-14] Only 63.3% of the our isolates from patients suffering from chronic urogenital infection in the from North of Palestine were enterotoxigenic, This study showed higher prevalence than our previous report,[5] which was 41.2% of test isolates. If other more recently described toxin genes in *S. aureus* were taken into account the prevalence might be higher than being reported.

It is important to mention that the detection of the toxin genes by genotypic techniques like PCR in Palestinian *S. aureus* isolates does not necessarily indicate the ability of these isolates

Table-II: Toxin gene profiles of 90 *S. aureus* isolates recovered from urogenital tract infected patients.

	No. of enterotoxigenic *S. aureus* isolates (90)					
Toxin gene	*Urine (51 isolates)*		*semen (39 isolates)*		*Total (90)*	
	+ve gene	-ve gene	+ve gene	-ve gene	+ve gene	-ve gene
sea	0	51	4	35	4 (4.4%)	86 (95.6%)
seb	6	45	6	33	12 (13.3%)	78 (86.7%)
sec	18	33	11	28	29 (32.2%)	61 (67.8%)
sed	0	51	0	39	0 (0%)	90 (100%)
see	0	51	0	39	0 (0%)	90 (100%)
Seb-c	3	48	3	36	6 (6.7%)	84 (93.3%)
Sed-e	4	47	0	39	4 (4.4%)	86 (95.6%)
Seb-d-e	2	49	0	39	2 (2.2%)	88 (97.8%)

to produce biologically active toxin or sufficient level of toxin to induce disease. To confirm the toxicity of these strains it is important to demonstrate the toxin levels that are produced by these enterotoxigenic strains.[13] The presence of a higher number of leukocytes in urine or semen samples in case of urogenital staphylococcal infection could be a factor that contributes genetic selection of strains that can produce superantigens. These toxins, others may have a damaging effect on that cell type. Those urogenital strains which carried enterotoxin genes may induce releasing of specific cytokines that may inhibit the efficiency of human immune response by their expressions; this may contribute to the persistence of *S. aureus* in urogenital tract and promote inflammation in these tissues or enhance the chronicity of this disease.

In the present study, the majority of the enterotoxigenic *S. aureus* isolates carried *sec* gene alone or in combination with other genes. These results were consistent with previous reports which showed that most human clinical enterotoxigenic *S. aureus* isolates harbored *sec* and *seb* genes.[15,16] However, these results were in contrast to other studies which showed that most human clinical enterotoxigenic *S. aureus* isolates carried *seg* and *sei* genes,[17,18] *seb* gene,[19,20] or *sea* gene.[5,10,11,21] This discrepancy in prevalence is possibly due to geographical differences, which may be related to different ecological origins of the isolated strains and the genes which have been detected. Our results in this study are different in many respects from our report that was published previously,[5] which has showed that all enterotoxigenic isolates detected previously harboured only one toxin gene. The possession of more than a single enterotoxin gene was reported, the multiple genes and their frequencies were variable in these studies.[4,10,11,14-16] The presence of multiple expressed toxins in a single *S. aureus* strain makes it less clear whether any single toxin is responsible for diseases.[22] In spite of this, the possession of multiple virulence genes supports a strong potential of the isolates to cause severe illness.

The role of *sea*, *seb*, *sec*, *sed* and *see* genes in the pathogenesis of urogenital tract infection is still unknown. However it is possible that urogenital infection can be caused by *S. aureus* strains at least lack these genes. Other newly detected genes may play a role in pathogenesis.

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