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Detection of *Staphylococcus aureus* Enterotoxins A to E from Clinical Sample by PCR

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Abstract: *Staphylococcus aureus* is one of the most significant pathogens causing nosocomial and community acquired infections. Among the secreted Staphylococcal virulence factors, there is a growing list of enterotoxins which can induce gastroenteric syndrome and toxic shock syndrome. Here we used PCR for the detection of genes encoding Staphylococcal enterotoxins A, B, C, D, E (SEA, SEB, SEC1, SED and SEE) of *S. aureus*. SEA-SEE were selected because they are 5 classically Described enterotoxins of *S. aureus* and because they were detected by Latex agglutination. We investigated 50 isolates of *S. aureus* drived from scar of patients. The presence of enterotoxin genes was found in 37 (74%) of total number of 50 isolates, for one or more enterotoxin genes. The PCR is more sensitive because it offers the possibility for determining enterotoxin as on a genotypic basis.

Key words: *Staphylococcus aureus*, enterotoxins, A-E, clinical sample, PCR

INTRODUCTION

Staphylococcus aureus is major human pathogens that produce a wide variety of exoproteins that cause various types of disease symptoms. Some *S. aureus* strains produce pyrogenic exotoxins, such as Staphylococcal enterotoxins (SEs) and toxic shock syndrome toxic 1 (TSST-1) (Sharma *et al*., 2000). Today, up to 17 SEs are known (Sergeev *et al*., 2004).

Beside the classically described SEs (SEA to SEE), only SEH, SEG, SEI have been proven to induce gastroenteric syndrome (Munson *et al*., 1998).

The 5 major serological groups of enterotoxins fall in 2 sub groups: SEB, SEC1, SEC2 SEC3 which have 66-98% amino acid sequence identity and SEA, SED and SEE, which have 53-81% amino acid sequence identity. The gene coding for TSST-1 has little sequence homology with SE or Streptococcal gynogenic exotoxin genes (Sharma *et al*., 2000; Klots *et al*., 2003; Munson *et al*., 1998). Although the toxins are structurally and functionally very similar.

Staphylococcal enterotoxins are low molecular weight proteins (MW 26, 900-29, 600), heat resistance is one of the most important physical and chemical properties of SEs, which means that biological activity of toxins remains unchanged even after thermal processing of food (Chapaval *et al*., 2006; Martin *et al*., 2004; Mcklauchlin *et al*., 1999).

They are encoded by mobile genetic elements including phages, plasmid and pathogenicity islands (Martin *et al*., 2004; Holeckova *et al*., 2002).

These enterotoxins have super antigenic activity; they stimulate T-cell proliferation, enhance endotoxic shock, suppress immunoglobulin production and are pyrogenic (Loir *et al*., 2003; Munson *et al*., 1998). Super antigen activity results from direct interaction of SEs with T-cell Antigen Receptors (TCR) and the MHC of Antigen Presenting Cells (APC). Super antigen toxins interact with many T-cells by recognition of specific vβ chains of the TCR (Loir *et al*., 2003; Mehrotra *et al*., 2000).

After ingestion of contaminated food toxins are resorbed in to the blood in the gastrointestinal tract, activate an emetic reflex, cause nausea, emesis, abdominal cramps and diarrhea (Munson *et al*., 1998; Chapaval *et al*., 2006).

Various methods have been developed for detecting enterotoxin production. Of this, it is Reversed Passive Latex Agglutination (RPLA) which is most commonly employed and is commercially available. In this test the enterotoxins are identified by antibodies specific for each of the enterotoxins. Cross reaction between SEB and SECs and between SEA and SEE have been reported (Sergeev *et al*., 2004). RPLA also depends on sufficient amount of toxin being produced in the absence of interfering bacterial products for successful detection. Toxin

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Table 1: Details of primers and amplicons

Primer name and size	Description	Nucleotide sequence	Gene location	PCR product size
SA-U (20)	Universal forward prime	r5-TGTATGTATGGAGGTGTAAC-3	--	
SA-A (18)	Reverse primer for sea	5-ATTAACCGAAGGTTCTGT-3	639-657	270
SA-B (18)	Reverse primer for seb	5-ATAGTGACGAGTTAGGTA-3	564-582	165
SA-C (25)	Reverse primer for sec	5-AATTGTGTTTCTTTTATTTTCATAA-3	485-510	102
SA-D (20)	Reverse primer for sed	5-TTCGGGAAAATCACCCTTAA-3	676-696	306
SA-E (16)	Reverse primer for see	5-GCCAAAGCTGTCTGAG-3	584-600	213

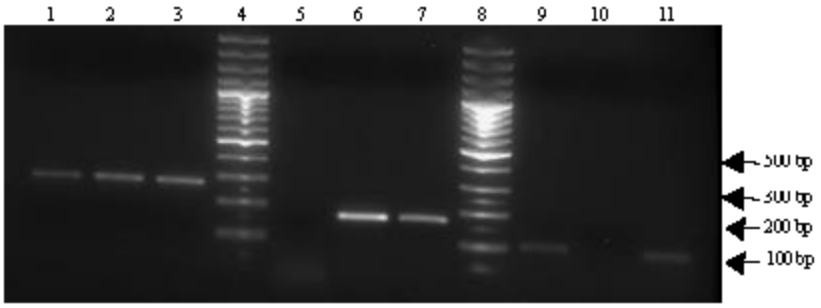

Fig. 1: Agarose gel electerophoresis patterns showing PCR amplification. The individual toxin gene products were characterized by comparing with standard molecular size marker. Lane 1-3 SEA (270bp); lane 4, 100bp size marker; lane 5 negative control for SEB; lane 6 and 7 SEB (165 bp); lane 8 100bp size marker; lane 9 and 11 SEC (102 bp); lane 10 negative control for SEC

production requires long (20 h) incubation periods and is also influence by culture conditions, pH, water activity and the substrate used (Sharma *et al*., 2000).

The PCR offers because bacterial enrichment is not required before a specific gene can be detected (Sharma *et al*., 2000; Hsiao *et al*., 2003; Rosec *et al*., 2002).

MATERIALS AND METHODS

Bacterial strains and culture media: Fifty *S. aureus* strains were studied. Strains were isolated from patient scars. Isolates were subcultured on Nutrient Agar (NA) and identified using the following tests: Gram staining result, the present of catalase-positive cocci in clamps, coagulase production and a characteristic hemolysis pattern when plated on sheep blood agar. In this study, the strains have used as positive controls, produced toxins (detected by ochtorlony test) (Imanifooladi *et al*., 2007) and negative control had no DNA in PCR reactions.

Isolation of genomic DNA: Total genomic DNA of *S. aureus* was isolated by modified phenol-chloroform method (Sambrook *et al*., 1989) lysates of colonies were prepared according to Sharma *et al*. (2000).

Oligonucleotide primers: The sequences and corresponding sequence location of oligonucleotide primers were used in this study are shown Table 1 that were ordered by from Faza Biotech (IRAN, Tehran). One forward primer common for all enterotoxin genes and 5 reverse primers were used (Table 1).

DNA amplification: PCR reaction were performed in a reaction buffer (10 X), $MgCl_2$ (4 mM) in a total volume of 50 μL, containing 1 μL (~1ng) of template DNA, 20-30 pm each of primers SA-U, SA-A, SA-B, SA-C, SA-D, SA-E and 0.2 mM of mix deoxy-nucleotide tri-phosphate and 1 unit of Taq DNA polymerase. Twenty-eight amplification cycles were performed as a following condition: Initial denaturation 94°C for 4 min, 94°C for 30 s, 50°C for 30 s, 72°C for 30 s with a final extension cycle of 10 min at 72°C, (standardization of PCR protocols have done as same as Sharma's study).

Detection of amplified DNA: A 10 μL aliquot of the amplified PCR product was analyzed on 2% TAE agarose gel containing 0.5 μg mL^{-1} ethidium bromide. Electrophoresis was performed at 80 V for 1 h. Gels were viewed by UV transillumination and photographe (Fig. 1).

Sequencing: The PCR production were sequenced by (Fazabiotech, Tehran, Iran) and had high homology with SEs genes of Staphylococcus aureus in Gen Bank (85-97%).

RESULTS

A total of 50 strains of patients scar , the presence of enterotoxin genes was found in 37 (74%).

Presence of SEA gene was detected in 9(18%), presence of SEB in 4 (8%), presence of SEC in 17 (34%), SEA together with SEC in 4 (8%), SEB together with SEC in 1(2%), SEA, SEB with SEC together in 2 (4%), isolates of *S. aureus* were detected. No one of isolates carried genes For SED, SEE.

Sequencing: The result of sequencing show that, our PCR product in enterotoxins A, B, C had 85-97% homology with BLAST in Gen bank.

DISCUSSION

The determination of Staphylococcal enterotoxins type has a long history of successful use in epidemiological studies in both clinical and environmental microbiology studies (Sharma *et al*., 2000). Many authors use PCR for detecting Staphylococcal enterotoxin genes (Tkacikova *et al*., 2003). All of them found a high variability (75-80%) in the presence of enterotoxin genes (Tkacikova *et al*., 2003). Some *S. aureus* strains produce one or more enterotoxigenic toxins including SEA-SEE and TSST-1 and these toxins represent the main cause of Staphylococcal food poisoning. It has been estimated that about 95% of these out breaks were due to classical SEs such as (SEA-SEE) (Sambrook *et al*., 1989; Omoe *et al*., 2002). The limitation of all genotypic tests is that the presence of the gene does not always necessarily mean that the toxin will be produce (Neill *et al*., 1990; Tsen *et al*., 1998). Some researches identified the presence on an SEC gene in two strains which did not produce detectable levels of SEC toxin when they used the SET-RPLA assay. This situation above and many others maybe due to low level production of enterotoxin below the threshold of detection for the immunological assay. The enterotoxin gene may not be expressed due to mutations either in the coding region or in regulatory region (Sharma *et al*., 2000). However, one major application of the immunological assay is the toxin typing of strains for epidemiological purposes it is no usually essential to know whether or not a gene is expressed (Chapaval *et al*., 2006).

In the reported by Omoe *et al*. (2002), analyzed 71 *S. aureus* isolates from various sources and 66 (93%) were found to be positive for on or more enterotoxin genes (Omoe *et al*., 2002).

In the reported by Klots *et al*. (2003), analyzed 93 *S. aureus*e isolates from patients at hospital, 44 (47%) were found to be positive by TaqMan PCR for one or more enterotoxin genes (Klots *et al*., 2003). The results of Mclanuchlin *et al*. (1999) stadies showed rate of presence enterotoxin genes (75%) was higher than we are reported (74%) (Mcklauchlin *et al*., 1999). Chapaval *et al*. (2006) was reported enterotoxin genes presence (68%) was lower than we are reported (Martin *et al*., 2004). Our studies show that presence of enterotoxin genes in scar is higher than the presence of enterotoxin genes in food. The relationship between *S. aureus* nasal carrier and Staphylococcal skin infection was significance (Imanifooladi *et al*., 2007). It seems that there is a relation between productivity of enterotoxin by *S. aureus* and the intensity of skin infections. In order to establish the fact it requires more studies, because enterotoxigenic *S. aureus* was present in more than 50% of patients with Psoriasis and Atopic dermatitis significantly correlate to enterotoxin production of isolated *S. aureus* strains (Tomi *et al*., 2005).

PCR is a rapid and extremely sensitive procedure, which is a very good tool for the detection of enterotoxin gene in clinical isolates of *S. aureus*. It can be used for specifying the Staphylococcal infection of the mammary gland and to speed up the diagnosis of the hazardous Staphylococcal strains (Tkacikova *et al*., 2003).

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