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Research Article

Virulence Genes in *Staphylococcus aureus* Strains Isolated from Different Clinical Specimens in an Iranian Hospital

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Abstract

Objectives: The aim of our study was to study the virulence markers in *Staphylococcus aureus* strains isolated from different clinical specimens in an Iranian Hospital.

Methods: From different clinical specimens such as wounds, blood and respiratory samples, *Staphylococcus aureu*s was isolated and identified based on standard methods 2014 to 2015. All the clinical specimens were analyzed for toxins genes by multiplex PCR .In addition, all the *S. aureus* isolates were evaluated for their antibiotic resistance pattern by disk diffusion method.

Results: A total of 300 *S. aureus* were isolated and identified. These isolates were screened for seven genes encoding virulence determinants a *setb, eta, sea, seb , sec , sed and tsst-1.*

270 isolates were positive for at least one of the genes. *Gene etb* (n = 30,%10), *eta* (n = 27,%9), *sea* (n = 120,%40), *seb* (n = 36, %12), *sec* (n = 3,%1), *sed* (n = 9,%3), *tsst-1* (n = 45,%15) and *femA* (n = 300,100%) were positive control.

The highest numbers of resistant isolates were found in relation to Methicillin (%85) (MRSA), Ampicillin (%72), and Cefepime (%52).

Conclusion: The increasing prevalence of community-acquired MRSA and its emerging antibiotic resistance is a serious problem for public health. According to the results genes sea was most of the abundant gene and sec gene was the least in *staphylococcus aureus* studied in this study.

Keywords: *Staphylococcal Enterotoxin (SE); Multiplex PCR; Exfoliative Toxins A and B*

Introduction

Staphylococcus species are the most common causes of nosocomial infections in the world. Among them, *Staphylococcus aureus* has the greatest capacity of pathogenicity between *Staphylococcus* species [1]. Therefore, particular attention should be paid to this bacterium. *S. aureus* produces a extensive spectrum of extracellular and cell wall-associated virulence determinants [2], which widely depends on producing a variety of extracellular toxins [3,4], including enterotoxins, toxic shock syndrome toxin 1 (TSST-1), exfoliative toxin (ET), hemolysins, and coagulase. *S. aureus* is one of the most commonly found pathogenic bacteria and hard to be eliminated from the human body [5,6]. According to the most current serological classification, six staphylococcal enterotoxin (SE) groups have been recognized; these groups are SEA,SEB, SEC, SED, and SEE and the recently characterized [7,8]. It should be noted that these groups are the main sources of food poisoning in humans and animals and can cause intensive intestinal peristalsis [9]. Apart from SE, TSST-1 produced from *S. aureus* is the cause of toxic shock syndrome which results from synergistic interactions of a variety of factors. Exfoliative toxin and pyrogenic toxin super antigen production enables *S. aureus* to cause staphylococcal scalded skin syndrome (SSSS), staphylococcal toxic shock syndrome and staphylococcal food poisoning. Experimental models indicate that the expression of receptors for fibrinogen and fibronectin is associated with endocarditis, whereas the presence of adhesins for sialoprotein, collagen and fibronectin is associated with arthritis and osteomyelitis. In this regard, TSST-1 produced from *S. aureus* is the cause of toxic shock syndrome among both humans and animals. In addition, it has been reported that ETs ETA and ETB are, either in conjunction or separately, involved in the occurrence of staphylococcal scalded-skin syndrome [9-12].

Over the last few decades, a massive increase in the emergence of *S. aureus* strains which are resistant to the antibiotics has been reported [13]. Staphylococcal infections are associated with increased morbidity and mortality all over the world. Over the past decade, antibiotic resistance pattern in *S. aureus* has highlighted the need for new antimicrobial agents. Although various antibiotic resistances particularly, Vancomycin-resistant *S. aureus* strains have been described in Iran, the clinical significance of this resistance pattern is very important at the present time.

For discussion of evaluation the resistance, the disk diffusion method has been conducted used for samples. The identification pathogenic strains of *Staphylococcus aureus* toxin using by Immunology methods as agglutination, radioimmunoassay, immune diffusion and ELISA assay considering Time-consuming, and lacking in specificity [14-21].

In this study, the PCR method was used to successfully and reliably detect SEs, TSST-1, and ETs. The primers used in this study were specifically designated to amplify *femA* as an internal positive control for each reaction; because provides proteins for cell wall metabolism and this products is essential for methicillin resistance (MRSA). However, the *femA* gene existence in most activity growing cultures of *S. aureus* [6,14,16].

The main objective of this investigation was to isolate, identify the *S. aureus* strains from different clinical specimens. Furthermore, these isolates were first characterized for their toxin gene profiles using particular primers [14].

Materials and Methods

All the clinical specimens were subjected to biochemical tests [22] for detection of *S. aureus*. All the clinical specimens were collected from Shahid Rajaii Hospital, Babol, Iran. This study was also approved by the ethic committee of Mazandaran University of Medical Sciences. All the clinical specimens' were evaluated for the presence of *femA* gene.

Antibiotics resistance pattern was performed using the disk diffusion method according to CLSI guidelines [23,24]. Antibiotics used in this study included: methicillin, ampicillin, cefepime, ceftriaxone, clindamycin, chloramphenicol, gentamisin, and cefazolin. All the antibiotic disks were purchased from Mast Company (United Kingdom).

DNA isolation

The total DNA, in this work, was isolated from 0.5 ml Luria broth (LB broth) culture, which was grown at 37˚C on shaker incubator overnight. DNA was isolated according to using Johnson study protocol, et al [14]. Briefly, cells were centrifuged at 1000 xg for 10 min, re-suspended in phosphate-buffered saline with 100 µl of lysostaphin (Sigma) per ml, and incubated at 37˚C for 30 minutes.

DNA from all preparations were subsequently extracted with phenol-chloroform and precipitated with ethanol. DNA samples were dissolved in TE buffer (10 mM Tris chloride-1 mM EDTA [pH 8.0]). All samples in order to confirm the proper concentration of DNA were measured by Nanodrop in the ratio of A260 to A280 and adjusted to a final concentration of 2, µg/ml with TE buffer according to A260 values [14,25]. Then all samples were analyzed for the presence of *femA* genes by PCR assay using specific primers which is specific gene in isolated *Staphylococcus aureus* [15].

The DNA was made in TE buffer used in 10 to 1000 ng (serial 10-fold dilutions) as templates for PCR reaction in this study.

Multiplex PCR conditions

The nucleotide sequences of all PCR primers and their respective amplified products are shown in Table1.

Gene	primer	DNA sequence (5'- 3')	Size (bp)	Reference
sea	1	GGTTATCAATGTGCGGGTGG	102	[6]
	2	CGGCACTTTTTTCTCTTCGG		
seb	1	GTATGGTGGTGTAACTGAGC	164	
	2	CCAAATAGTGACGAGTTAGG		
sec	1	AGATGAAGTAGTTGATGTGTATGG	451	
	2	CACACTTTTAGAATCAACCG		
sed	1	CCAATAATAGGAGAAAATAAAAG	278	
	2	ATTGGTATTTTTTTTCGTTC		
eta	1	GCAGGTGTTGATTTAGCATT	93	
	2	AGATGTCCCTATTTTTGCTG		
etb	1	ACAAGCAAAAGAATACAGCG	226	
	2	GTTTTTGGCTGCTTCTCTTG		
Tsst-1	1	ACCCCTGTTCCCTTATCATC	326	
	2	TTTTCAGTATTTGTAACGCC		
femA	1	AAAAAAGCACATAACAAGCG	132	
	2	GATAAAGAAGAAACCAGCAG		

Table1: *Nucleotide sequences and anticipated sizes of PCR products for the S. aureus gene-specific oligonucleotide primers used in this study.*

The final optimized PCR reaction consists of 5 ml of 10x reaction buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl), 1.5 mM $MgCl_2$, 20 pmol (each) of *sea, seb, sec and femA*, 50 pmol for *eta* and 20 pmol each for *etb, tst* primers; and also, 40 pmol of *sed* primer, 2.5 U of *Taq*DNA polymerase (AmpliTaq DNA polymerase; Perkin-Elmer), and 10 to 1000 ng of template DNA was applied in the optimized PCR reaction. The volume of this mixture was adjusted to 50 ml using sterile water.

To amplify DNA, an ABI thermocycler with the following thermal cycling profile was applied:

An initial denaturation at 94°C for 10 min followed by 35 cycles of amplification (denaturation at 94°C for 1 min, annealing at 58°C for 1 min for *femA* and another genes at 55°C for 2 min, and extension at 72°C for 1 min), ending with a final extension at 72°C for 5 min [6,14,26].

Results

300 clinical specimens were collected from rom patients who referred to rajaee hospital during one year. Of the 300 patients 43% were female and 57% % were male and the predominant age group was 25 - 30 years (Figure 1).

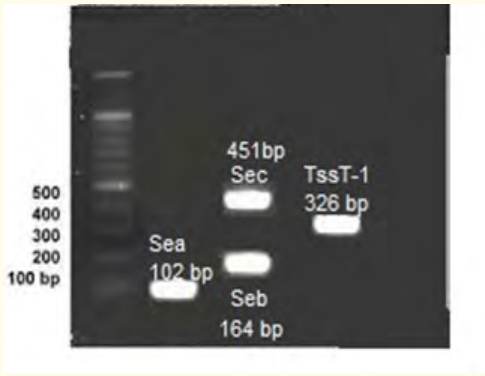

Figure 1: *Agarose gel electrophoresis patterns showing multiplex PCR of sea, seb, sec and Tsst-1 genes for the S. aureus.*

A total of 300 *S. aureus* were isolated and identified. These isolates were screened for seven genes encoding virulence determinants (*etb, eta, sea, seb, sec, sed and tsst-1*).

270 isolates were positive for at least one of the genes. *Gene etb* (n = 30, %10), *eta* (n = 27, %9), *sea* (n = 120, %40), *seb* (n = 36, %12), *sec* (n = 3, %1), *sed* (n = 9, %3), *tsst-1* (n = 45, %15) and *femA* (n = 300, 100%) were positive control.

The highest numbers of resistant isolates were found in relation to Methicillin (%85) (MRSA), Ampicillin (%72), Cefepime (%52) and also, the highest sensitive antibiotics was found in Vancomycin (%80), Chloramphenicol (%70), Gentamicin (%67).

Discussion

Many countries, Iran with no exception, has experienced a steady increase in antibiotic resistance among *S. aureus* strains isolated from various infectious disease during the last decade [2,27]. The analysis of antibiotic resistance revealed that 70% of the *S. aureus* isolates were at least resistant to one antibiotics which is similar to other findings in Iran [4,27]. This observation supports the findings of Johnson, Cha and Teyhooet., *et al*. who reported a resistance rate of 94% *S. aureus* isolated to 83% in neighboring countries [28].

S. aureus has been suggested to contribute to the severity of the infection because of the *S. aureus* virulence factors that are involved in colonization and the subsequent infection. Staphylococcal adhesions, toxins, and genes involved in its spreading may be involved in the development of various infections. Therefore, to understand the role of some genes present in *S. aureus* strains, the presence of various virulence genes coding for adhesions.

Toxins, or other products was determined in *S. aureus* strains isolated from different clinical specimens. In this study, we designed and described rapid diagnostic protocols to detect genes of enterotoxins A to C, ETA, ETB, and TSST-1 in *S. aureus*. The prevalence of the detected various virulence genes among the studied isolates are summarized in Figure 2.

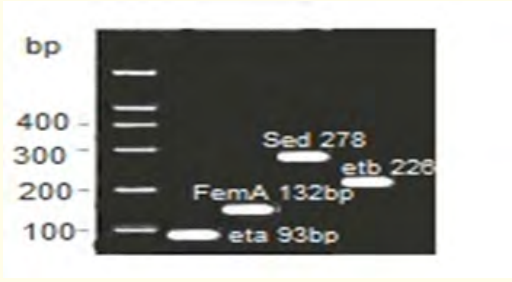

Figure 2: *Agarose gel electrophoresis patterns showing multiplex PCR of sed, eta, etb and FemA genes for the S. aureus.*

In this study, the abundance of *tsst-1* gene was found to be %15; This observation supports the findings of 20% in Tabriz Hospital, Tabriz, Iran [16,29].

In many studies, the sensitivity to various antibiotics like (e. g. vancomycin, chloramphenicol, gentamicin, cefazolin) has been analyzed and their reports are approximately similar to this study's [27,30-33].

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