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Research Paper

Investigating prevalence of pathogenic genes (ETA and TSST-1) in Staphylococcus aureus isolated from different wards of the hospitals by PCR method

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Abstract

Staphylococcus aureus is the most common pathogenic organisms in the hospitals and communities' infections. It is responsible for more than 80 percent of infectious diseases. The purpose of the present paper is to determine the incidence of Staphylococcus pathogenic genes isolated from different wards of hospitals by PCR method. This study included 61 Staphylococcus aureusisolates collected from different wards hospital, between 2011 and 2012 in University of Kurdistan (Toohhid and Besat hospitals). All isolates were previously identified as Staphylococcus aureusby a standard microbiological procedure. It isolates were incubated at 37Ċ for 24h on blood agar; single colonies were tested with tube and slide coagulase, catalase tests and growth on Manito salt agar. Following genomic DNA extraction, the presence of ETA, TSST-1 genes was analyzed by PCR.61 strains of Staphylococcus aureushave been isolated from different wards of the hospital. Frequency of tst gene was 81% and eta gene was 47%. Moreover, frequency of strains with both eta and tst genes was 40%. Results of the present paper indicate that the prevalence of Staphylococcusaureus results on prevalence of eta and tst genes, and this is a matter of concern.

Keywords: *TSST-1; ETA; Staphylococcus aureus; PCR Method; Penicillin.*

1. Introduction

In 1950, the emergence of antibiotic-resistant strains provided a better understanding of epidemiology of Staphylococcus aureus disease. At first, emergence of penicillin was very helpful for treating Staphylococcus aureus infections. However, a considerable number of penicillin-resistant strains were obtained from Staphylococcus aureus infections in 1946[1]. Pathogenesis of Staphylococcus aureus could be because of its producing pyrogenic toxins. In fact, secretion of this toxin significantly stimulates T-lymphocytes, which are called superantigens (SAgs) [2], [3].So So far, far, 15 types of super antigens in Staphylococcus aureus have been recognized and Staphylococcal enterotoxin A-M, exfoliative toxin A, B, and toxic shock syndrome toxin (TSST-1) are some samples of it. TSST-1 is a protein with molecule weight approximately equal to 24kDa and isoelectric point 6.8-7.2, which are coded by tst gene of Staphylococcus aureus. Release of TSST-1 in blood flow results in acute clinical conditions such as toxic shock syndrome (TSS), syndrome of sudden death of infants, and Kawasaki syndrome's gene is presented in more than 70 percent of Staphylococcus aureus strains isolated from patients with TSS. TSS is characterized by skin rashes, high fever, severe fall in blood pressure, and muscle pains. If TSS is not treated up to one hour after the emergence of symptoms, it could result in a fatal shock. Two third of TSS is related to using tampons; however, some cases are related to localizing infections, surgery wounds and insects bites [4-6]. TSST-1 is one of the important factors related to

the virulence of this bacterium; it is one of the pyrogenic toxins superantigens that has important effects on its host [7]. Exfoliative toxin is an extra cellular protein that causes staphylococcal scalded skin syndrome (SSSS) in children and teenagers. Three isoforms of exfoliative toxin have been isolated from human; the maximum frequency belongs to ETA and ETB, and the minimum frequency belongs to ETD. However, ETC has been isolated from animal infections and is not pathogenic to humans [8]. In this study, we are investigating prevalence of pathogenic genes tst and eta isolated from different wards of the hospital by PCR method.

2. Methodology

2.1. Sample collecting

This study included 61 Staphylococcus aureus isolates collected from different hospital wards, between 2011 and 2012 in Kurdistan University of medical sciences (Toohhid and Besat hospitals). All isolates were previously identified as Staphylococcus aureus by a standard microbiological procedure [9]. It isolates were incubated at 37Ċ for 24h on blood agar and single colonies were tested with tube and slide coagulase, catalase, DNase tests, and growth on Mannitol salt agar. For confirmation of isolates as Staphylococcus aureus we used nuc gene codifies the nuclease-resistant to heat as a marker [10] After extracting DNA, five micro liters of each specimen with two micro liters diluted loading to dye was run over 1% agarose gel, and electrophoresis was done to ensure the presence of DNA.

2.2. Staphylococcus aureus DNA extraction

DNA Cinna Pure kit manufactured by Sina Gene was used to extract genomic DNA of the isolations of Staphylococcus aureus, After extracting DNA, 5 ml of each specimen with 2 µl diluted loading dye was run over 1% agarose gel and electrophoresis was done to ensure the presence of DNA. The extracted DNA should be kept in the refrigerator temperature of 4 Ċ. Some bacteria`s DNA can be kept in the refrigerator temperature of -20 Ċ but freezing can cause DNA to break. The long term maintenance is possible under the absolute ethanol or isopropanol in the -20 Ċ [11].

2.3. PCR amplification of nuc gene

DNA template was prepared, purified and stored until needed at -20Ċ[12]PCR amplification of the nuc gene was performed to confirm Staphylococcus aureus. PCR was performed with 2µl extracted template DNA, 2µl nuc primers (5'AGTTCAGCAAATGCATCACA-3', 5'- ACGCAA GCC TTG ACG AAC TAA AGC-3') [10], and 11µLof master mix (polymerase Taq enzyme,$Mgcl_2$,,dNTP, $SO_4(NH_4)_2$, TrisHCI, Tween – 20) and10µl deionized water a final volume of 25µL.The thermal cycling program was as follows: initial denaturation (5 min at 94℃); followed by 30 cycles of denaturation (60 Sec at 94℃), annealing (60 Sec at 55℃), and extension (60 Sec at 72℃); and a single extension (7min at 72℃).

2.4. PCR amplification of tst gene

Detection of tst gene in S.aureus strains was carried out using PCR as described previously (Johnson & Tyler, 1993). PCR was performed with 2 µL extracted template DNA, 2 µL tst primers (5'- ACCCCTGTTCCCTTATCATC-3', 5'-TTTTCAGTATTTGTAACGCC-3' [13],and 12.5µl master mix (polymerase Taq enzyme, $Mgcl_2$, dNTP, $SO_4(NH_4)_2$,TrisHCI, Tween – 20) and 8.5µl deionized water in a final volume of 25 µl. The thermal cycling program was as follows: initial denaturation (5 min at 94℃); followed by 30 cycles of denaturation (60 sec at 94℃), annealing (60 sec at 55℃), and extension (60 sec at 72℃); and a single extension (10min at 72℃).

2.5. PCR amplification of eta gene

Detection of tst in S.aureus strains was carried out using PCR as described previously [14] PCR was performed with 2 µl extracted template DNA, 2µL eta primers (5'- ATGGCAGCATCAGCTTGATA-3', 5'-TTTCCAATAACCACCCGTTT-3'[13]and 12.5µl master mix (polymerase Taq enzyme, $Mgcl_2$, dNTP, $SO_4(NH_4)_2$, TrisHCI, Tween – 20) and 8.5µl deionized water in a final volume of 25 µl. The thermal cycling program was as follows: initial denaturation (5 min at 94℃); followed by 30 cycles of denaturation (60 Sec at 94℃), annealing (60 Sec at 55℃), and extension (60 Sec at 72℃); and a single extension (10min at 72℃).

3. Results and Discussion

As shown in Table 1 the frequency of pathogenic genes that encode eta, tst were high in isolate from pediatrics in compare with other wards. There were 61 strains isolated that include 35 clinical strains, 6 strains personnel colonized, and 20 strains hospital environment colonized strains as shown in Table 2.

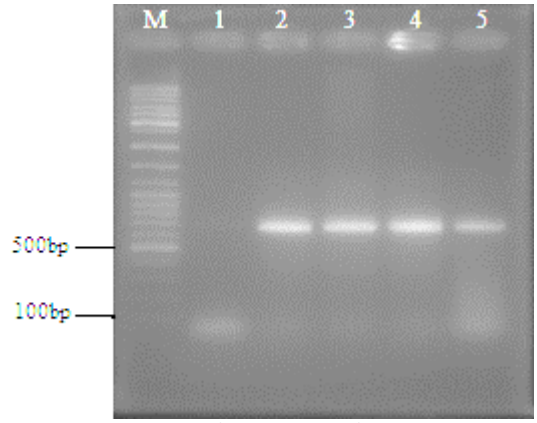


Fig. 1: Electrophoresis of PCR Products for nuc gene.

Line M: marker, molecular weight (100-1000 bp) Line 1: strain of negative control, without nuc gene 2, 3, 4: positive samples with nuc gene (680 bp), Line 5: strain of positive gene (ATCC 33591, S. aureus), with nuc gene Lines

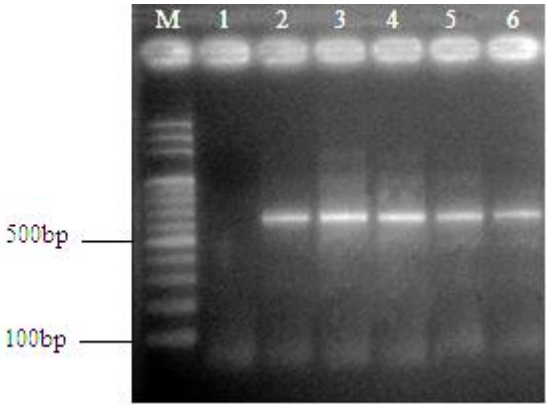


Fig. 2: Electrophoresis of PCR Products for eta gene.

Line M: marker, molecular weight (100-1000 bp) Line 1: strain of negative control, without eta gene Lines 2, 3, 4, 5: positive samples with eta gene (650 bp), Line 6: strain of positive gene (ATCC 33591, S. aureus), with eta gene

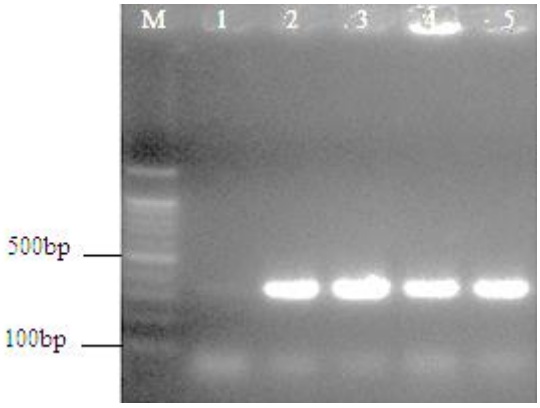


Fig. 3: Electrophoresis of PCR Products for tst gene.

Line M: marker, molecular weight (100-1000 bp) Line 1: strain of negative control, without tst gene 2, 3, 4: positive samples with tst gene (314 bp), Line 5: strain of positive gene (ATCC 33591, S. aureus), with tst gene Lines

Table 1: Prevalence of eta and tst Genes among Different Wards of the Hospital

Wards	Sample	Isolates(number of strains)	tst (positive strains)	eta (positive strains)
ICU	Urine,Trachea, Blood	13	8(61%)	3(23%)
Pediatric	Blood, Nasal swab.	8	8(100%)	5(62.5%)
Surgery	CSF, laryngoscope	6	4(66%)	3(50%)
Emergency	Patient bed	5	3(60%)	1(40%)
Operation	Nasal swab	4	4(100%)	2(50%)
Infant	Blood	4	3(75%)	1(25%)
Internal	Blood	4	4(100%)	2(50%)
Infection	Nasopharynx swab	3	3(100%)	3(100%)
Neurology	Urine	2	1(50%)	2(100%)
PICU	Secretion	2	1(50%)	1(50%)
Orthopedic	Patient bed	2	2(100%)	1(50%)
NICU	Blood	1	1(100%)	Without gene
Cardiac	Urine	1	Without gene	Without gene
Burning	Biopsy	1	Without gene	1(100%)
Oncology	Blood	1	1(100%)	Without gene
CCU	Hand	1	1(100%)	Without gene
Laboratory	Hand	1	1(100%)	1(100%)
Infection	Anesthesia machine	1	1(100%)	Without gene
Neurology	Trolley	1	1(100%)	1(100%)
Total number=61				

Table 2: Prevalence of eta and tst genes among Clinical Sample, Hospital Environment and Personnel in Hospitals (Positive Strains)

Groups Number of strains	Hospitals		tst gene(%)	eta gene(%)	tst and eta(%)
	T	B			
clinical sample 35	15	20	29 (82)	16 (45)	14 (40)
hospital environment 20	5	15	15 (75)	8 (40)	8 (40)
Personnel 6	4	2	5 (83.3)	2 (33.33)	3 (50)
Number of strains = 61					

T=Toohhid hospital B= Besat hospital

Staphylococcus aureus is one of the most important human pathogens that has been a causative factor of acquired infections in community and hospital levels during the last decades [3], [14]. As major virulence factors in Staphylococcus aureus, TSST-1and ETA are pyrogenic toxins that have been implicated in host colonization, invasion of damaged skin and mucus and evasion of host defense mechanisms. [15], [16] The coordinated expression of S. aureus secreted and cell wall-associated virulence factor is regulated by a complex network, including the quorum-sensing (QS) system agr and the well characterized virulence gene regulators [17]. In comparison with other methods, PCR is a proper, sensitive and cheap method for recognizing toxigenic genes [14] . Therefore, PCR method has been used in this study to recognize the rate of eta and tst genes. In overall, 61 strains of Staphylococcus aureus have been isolated from different wards of the hospital such as Pediatric ،Surgery ،emergency ،Operation ،Infant ، internal ، Infection ، Neurology ، PICU، orthopedic، NICU, Cardiac, Buring, Oncology، CCU ،laboratory, anesthesia machine, ICU and trolley. 35 strains are isolated from patients, 6 strains from personnel and 20 strains from the hospital environment. In this study, frequency of tst gene was 81% and eta gene was 47%. Moreover, frequency of strains with both eta and tst genes was 40%. In this study, the most samples were collected from ICU, Pediatric wards. Results indicate more prevalence of tst and eta genes in these wards, so this issue would endanger of public health and Hygiene. Our results was in agreement with Shi studied that has done in children with impetigo, prevalence of eta gene in strains methicillin-resistant staphylococcus aureus (MRSA) and strains of methicillin sensitive (MSSA) were investigated. According to the results of this study, 61.5% of MRSA and 90.6% of MSSA strains were carriers of eta gene[18]In the other hand, some other reports was lower frequency of tst gene was 48%[19]The discrepancy in frequency in different geographical indicate dissemination of pathogenic organisms and it seems that we are exposure with high pathogenic organisms. This information in critical for nosocomial prevention committee. In fact, another research was done in Czech and showed that 48 strains out of 59 isolated strains were carriers of eta gene. In this research, 40 strains were isolated from sick children, 10 strains from nurses' hands and 5 strains from the equipment [20] Another research was done in 2005; in this research 103 strains of Staphylococcus aureus were isolated from community, hospital and patients with granulomatous. 12 strains out of 51 strains isolated from community, 5 strains out of 34 strains isolated from hospital and 4 strains out of 16 strains isolated from patients with granulomatous reported presence of tst gene[21]In the present research, 29 strains out of 35 strains isolated from the patients, 5 strains out of 6strains isolated from the personnel and 15 strains out of 20 strains isolated from the equipment included tst gene. Moreover, 16 strains out of 35 strains isolated from the patients, 4 strains out of 6 strains isolated from the personnel and 8 strains out of 20 strains isolated from the equipment included eta gene.

4. Conclusion

The results showed that prevalence of Staphylococcus aureus that was carriers of eta and tst genes was a concerning issue. Report of this isolation in community, especially for children, old people and other cases with less immunity was hazardous. By considering a high value of colonization of this bacterium among healthy people that could arrive at 50-60 percent in nasopharynx area and 5-30 percent in skin and hair, this issue would be considered as a significantly important issue.

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