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Prevalence of Exfoliative and Toxic Shock Syndrome Toxin Genes in Methicillin-resistant *Staphylococcus aureus* Strains Isolated from Clinical Specimens in Makkah, Saudi Arabia

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Short Communication

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ABSTRACT

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains possessing virulence genes encoding such toxins as exfoliative toxins (ETs), toxic shock syndrome toxin-1 (TSST-1), is worrying, especially in relation to the increasing frequency of nosocomial infections. The present study aimed to determine the prevalence of genes encoding ETs and TSST-1 in MRSA isolates by polymerase chain reaction (PCR). The results showed that out of 88 investigated MRSA isolates, *tst* and *etb* toxin gene were found in 3 (3.4%) and 2 (2.3%) respectively, while none *eta* toxin genes were detected. It was concluded that the incidence of ET and TSST-1encoding genes among MRSA isolates in Makkah is lower or near to the global prevalence.

Keywords: MRSA; exfoliative toxins; toxic shock syndrome toxin-1; mecA.

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1. INTRODUCTION

MRSA has been steadily increasing in the world and nosocomial infections are now a serious problem because of the limited number of effective antibiotics available for treatment [1]. The resistance of the organism is due to the acquisition of the methicillin resistance gene *mec*A coding for the low-affinity penicillin-binding protein (PBP2A) [2]. In addition, *S. aureus* produces numerous virulence factors that contribute to its ability to cause infections. Enterotoxins, toxic shock syndrome toxin 1(TSST-1), exfoliative toxin (ET), haemolysins and coagulase are among various virulence factors produced by *S. aureus*. The enterotoxins, and TSST-1, belong to a family of superantigens [3]. TSST-1 is a major virulence factor in toxic shock syndrome (TSS), staphylococcal scarlet fever, and neonatal toxic shock-like exanthematous diseases [4]. ETs are associated with scalded skin syndrome. There are three serological forms of staphylococcal ETs (ETA, ETB, and ETD), all of which cleave human desmoglein1. ETs involved in human diseases consist of 2 types, ETA and ETB [5]. Both toxins cause exfoliation of the epidermis without necrolysis or inflammatory response of the skin [5]. Some studies suggested the possibility of MRSA strains to carry genes encoding ETs [6,7]. The ability of *S. aureus* strains to produce ETs, and/or TSST-1 is an important property with various clinical implications because determination is still mainly based on immunological methods for toxin detection which are time and labor-consuming. Furthermore, these methods depend on the concentration of toxin expressed and thus can be negatively influenced by various factors [8]. Molecular data regarding MRSA carrying genes encoding ETs and TSST-1 in Saudi Arabia are generally not available. The present study aimed to determine the prevalence of genes encoding ETs and TSST-1 in clinical MRSA isolates by PCR in Makkah Saudi Arabia.

2. MATERIALS AND METHODS

A total of 88 MRSA isolates were obtained within two years ago from five main tertiary care hospitals in Makkah. All the strains were analysed for their *mec*A, *eta*, *etb* and *tst* genes by PCR. DNA was extracted according to the procedure mentioned by Bollet et al. [9]. Briefly a single colony was taken from and cell suspensions were centrifuged then cell pellets were washed with 1 ml of TE (10 mM Tris, pH 8, 10 mM EDTA). After addition of 50 u1 of 10% SDS, the mixture was incubated for 30 min at 65ºC. Supernatants were transferred into micro tubes which were then placed in a microwave oven and heated three times for 1 min at 750 W. The pellets were dissolved in 200 ul of TE and were extracted with an equal volume of phenol / chloroform / isoamyl alcohol (25:24:1) for 15 min. The aqueous phase was recovered by centrifugation for 20 min and precipitated with ethanol and then resuspended with 50 ul TE. The resistance of *S. aureu*s to methicillin was confirmed by detection of the *mec*A gene to ensure the fact that only MRSA strains were included in the study, then followed by detection of the of the *eta, etb* and *tst* genes. Fifty µl PCR mixture containing 8 µl of DNA template, 1 µl (100 pmol) of each primer and a 25 µl of Taq PCR Master (Promega Company) was prepared. Amplification was performed using Mastercycler PCR machine (Eppendorf, Germany). For the *mec*A, The thermal cycling conditions were as follows: an initial denaturation step at 94ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 53ºC for 30 s and extension at 72ºC for 1 min. While for *eta*, *etb*, and *tst* genes, (multiplex) the thermal cycling conditions consisted of initial denaturation for 5 minutes at 94ºC and 35 cycles at 94ºC for 5 minutes for denaturation, 55ºC for 1 minute for annealing and 72ºC for 1 minute for extension. Final extension was performed at 72ºC for 10 minutes. The PCR products were analyzed by electrophoresis in 1.5% agarose gels, 100 bp DNA ladder was included in each run and DNA bands were viewed under UVP BioDoct It Imaging System after staining with ethidium bromide (2 g/ml).

3. RESULTS AND DISCUSSION

The present study used a multiplex PCR-based protocol to detect the genes for ETA, ETB, and TSST-1 toxins the in DNA extracted from clinical MRSA isolates. The resistance of *S. aureu*s to methicillin was confirmed by detection of the *mec*A gene in accordance with the fact that only MRSA strains were included in the study. The emergence of isolates possessing a certain spectrum of virulence genes is worrying, especially in relation to the increasing frequency of nosocomial MRSA strains. In the present study, out of 88 MRSA isolates collected from 5 hospitals in Makkah, 3 (3.4%) were positive for *tst*, 2 (2.3%) were positive for *etb* toxin gene, (Fig. 1). None of the isolates analyzed amplified of the classical *eta* toxin genes.

Table 1. Primers of genes used in the study

Gene	Primer	Oligonucleotide sequence (5′→3′)	Size (bp)	Reference
*mec*A	mecA-P4	TCCAGATTACAACTTCACCAGG	162	[10]
	mecA-P7	CCACTTCATATCTTGTAACG		
tst	TSST-1	ATGGCAGCATCAGCTTGATA	350	[11]
	TSST-2	TTTCCAATAACCACCCGTTT		
eta	ETA-1	CTAGTGCATTTGTTATTCAA	119	[11]
	ETA-2	TGCATTGACACCATAGTACT		
etb	ETB-1	ACGGCTATATACATTCAATT	200	[11]
	ETB-2	TCCATCGATAATATACCTAA		

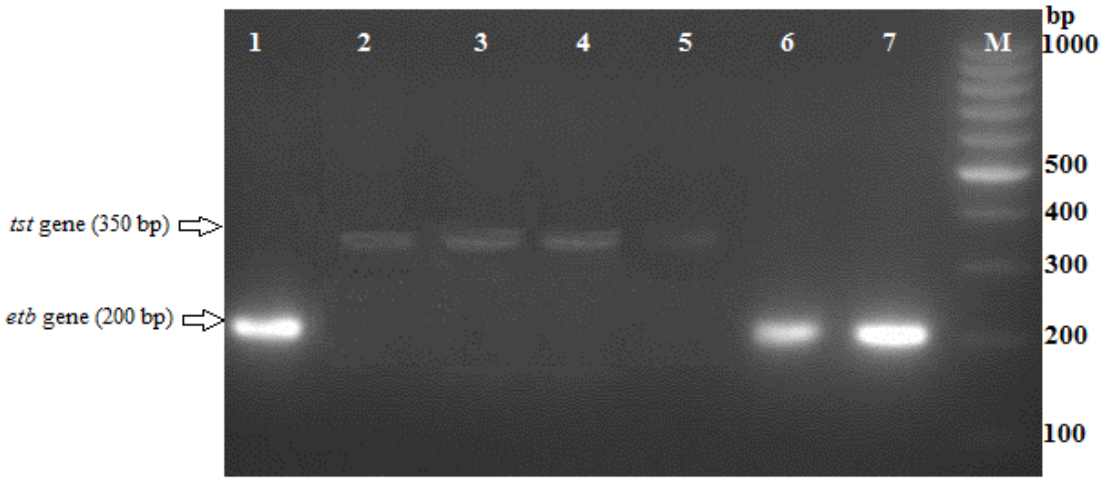

Fig. 1. *tst*, and *etb* encoding genes after PCR on 1.5% Agarose gel electrophoresis
Lane 1: etb positive control, Lane 2: tst positive control, Lane 3-5: isolates possessing tst genes, Lane 6-7: isolates possessing etb genes, Lane M: 100-bp DNA ladder

The TSST-1 often occurs together with septic shock and toxic shock syndromes and exfoliative toxins are characteristic for isolates causing staphylococcal scalded skin syndrome [12,13]. Similarly Taj et al. (2014) [14] reported *etb* gene in 1 (0.86%), *eta* gene in 2 (1.73%) and *tst* gene in 4 (3.47%) in MRSA isolates from clinical isolates while Tsen et al. [15] and El-Ghodban et al. [16] identified 3(4.8%) and 3(7.5%) strains of *S. aureus* from clinical sources as *tst*-carrying strains respectively. In contrast, Dagı et al. [17] reported *tst* 29 (27.9%), *eta* toxin gene 3 (2.9%) and detected none *etb* toxin gene. Previously it has been suggested that the *tst* gene is more prevalent in MRSA than in methicillin-susceptible *S. aureus* [18-20]. The low rates of positive isolates of ET encoding genes, found here are in accordance with the results of other investigations on MRSA strains producing toxins [20-24]. Generally, it could be concluded that the ETs and TSST-1encoding genes among MRSA isolates in Makkah, is low regarding the global prevalence.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The author hereby declares that all experiments have been according to the Institutional Ethics Committee of Medical sciences in Umm Al-Qura University, Saudi Arabia.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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