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Original Research Article

DETECTION OF COMMUNITY ACQUIRED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* BY POLYMERASE CHAIN REACTION

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered to have emerged from *S. aureus* through the acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*), which carries the *mec*A gene for methicillin resistance. Community-acquired *Staphylococcus aureus* (CA-MRSA) strains carry one of SCC*mec* types (IV or V). In addition they carry Panton-Valentine leukocidin (PVL) gene. The aim of this study was to evaluate the prevalence of CA-MRSA in Makkah hospitals by detection *mec*A, SCC*mec* types (IV and V) and PVL genes. One hundred bacterial isolates were confirmed to carry *mec*A gene. CA-MRSA was detected in 44% of these MRSA isolates, 30% were SCC*mec* type IV; 14% were SCC*mec* type V and 21% were harbored the PVL gene. Molecular methods are useful for diagnosis and typing of MRSA pathogens. CA-MRSA was found to be high in Makkah hospitals and hence the infection-control guidelines for of CA-MRSA are necessary to be improved.

Key Words: CA-MRSA, HA-MRSA, *mec*A, MRSA, PCR, PVL, SCC*mec*

INTRODUCTION

MRSA, has been a major cause of nosocomial infections since the 1960s [1]. In addition, since the period from 1997 to 1999, CA-MRSA has also become a major concern worldwide. The prevalence of CA-MRSA has been reported in different regions of Saudi Arabia in the last years [2, 3]. CA-MRSA infections in healthy individuals without established risk factors have now been documented in the community in Saudi Arabia [2]. MRSA is considered to have emerged from *S. aureus* through the acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*), which carries the *mec*A gene for methicillin resistance [4]. It has shown a steady increase, and is becoming a significant public-health concern. CA-MRSA is associated with many diseases, example skin and soft tissue infections, necrotizing pneumonia and sepsis [4]. MRSA strains have been proved genetically distinct with respect to the SCC*mec* type they contain, and most healthcare acquired (HA-MRSA) strains carry one of three types of SCC*mec* type I, II, or III (34–67 kb) whereas most CA-MRSA strains carry one of SCC*mec* type IV or V (24 kb) [5, 6]. CA-MRSA often carries genes for PVL, a harmful toxin which destroys bacterium-engulfing immune cells and also respiratory tissue [7, 8]. However, it has been concluded that presence of PVL, together with SCC*mec* type IV or V, is a genetic marker for CA-MRSA [9]. Various molecular typing techniques have been developed to investigate the spread and evolution of MRSA. The most commonly used techniques include pulsed-field gel electrophoresis, multi locus sequence typing and SCC*mec* typing [10]. The present study evaluated the prevalence of CA-MRSA by detection *mec*A, SCC*mec* types (IV and V) and PVL genes in Makkah hospitals by using polymerase chain reaction (PCR).

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MATERIALS AND METHODS

Bacterial Isolates

This study carried out during March-May 2014. A total of 206 *S. aureus* isolates were obtained within two years ago from five main tertiary care hospitals in Makkah. The identification of *S. aureus* was performed by standard laboratory methods (Gram staining, growth and fermentation on mannitol agar, catalase, coagulase test and DNase test agar). *S. aureus* clinical isolates were detected as MRSA (oxacillin-resistant) using the Oxoid penicillin binding protein (PBP2′) latex agglutination test, and the findings were confirmed by the oxacillin-salt agar screening test, according to NCCLS guidelines [11]. All confirmed MRSA were analysed for their *mec*A, SCC*mec* types (types IV & V) and PVL genes by PCR.

DNA extraction and PCR assay

DNA was extracted by taking a single colony from a nutrient agar plate (Oxoid) that had been incubated overnight. Cell suspensions were centrifuged at 4,500 rpm for 5 min at 4°C. Cell pellets were washed with 1 ml of TE (10 mM Tris, pH 8, 10 mM EDTA) and were re-suspended in 100 ul of TE. After addition of 50 u1 of 10% SDS, the mixture was incubated for 30 min at 65°C. The lysates were centrifuged and supernatants were removed. The micro tubes 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 re-suspended with 50 ul TE [12].

The primers used in this study were obtained from IDT Integrated DNA technologies (IDT, Belgium) and shown in Table 1. PCR conditions is shown in table 2. PCR was carried in 50µl PCR reaction volumes containing 8µl of template DNA, 1µl (100 pmol) of each primer and a 25µl of Taq PCR Master Mix containing 100mM Tris-HCl, 500mM KCl at pH 8.3 at 20°C, 1.5mM MgCl2, 200M each deoxyribonucleoside triphosphate and 0.025U Taq polymerase (Qiagen, USA). Amplification of DNA was performed using Master cycler Personal Thermal Cycler (Eppendorhoff, Germany) (Table 2). 25µl of the PCR products were mixed 10µl of loading dye and analyzed by electrophoresis in 1% agarose gels (for 35 minutes at 90 V using 5 X TBE running buffer. Also, 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).

Table 1: Primers of genes used in the study

Primer	Primer Sequence (5'_3')	Product size (bp)	Reference
*mec*A-P4	TCCAGATTACAACTTCACCAGG	162	[13]
*mec*A-P7	CCACTTCATATCTTGTAACG		
SCC*mec* type IV-F	TTTGAATGCCCTCCATGAATAAAAT	450	[14]
SCC*mec* type IV-R	AGAAAAGATAGAAGTTCGAAAGA		
SCC*mec* type V-F	GAACATTGTTACTTAAATGAGCG	325	[15]
SCC*mec* type V-R	TGAAAGTTGTACCCTTGACACC		
Luk-pvl- F	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	[16]
Luk-pvl- R	GCATCAAGTGTATTGGATAGCAAAAGC		

Table 2: PCR thermocycling conditions

PCR	Temperature (°C) / Time					Cycle No
	Initial denaturation	Cycling condition Denaturation	Annealing	Extension	Final extension	
PVL *mec*A	94/5min	94/40sec	53/40sec	72/1 min	72/10 min	35
SCC*mec* IV SCC*mec* V	95/5min	95/1min	55/1min	72/1.5 min	72/10 min	35

RESULTS

Only 100 (48.5%) out of 206 bacterial isolates were confirmed as MRSA based on the presence of *mec*A gene. The results of mecA and MRSA SCC*mec* typing genes are shown in Table 3 and figure 1. The results of PVL genes are shown in table 3 and figure 2.

Table 3: *SCCmec* types and PVL among MRSA isolates

Genes	Confirmed MRSA isolates		
	Sccmec IV	Sccmec V	Total
Sccmec (100)	30 (30%)	14 (14%)	44(44%)
PVL(21)	11 (52.5%)	2 (9.5%)	13 (62%)

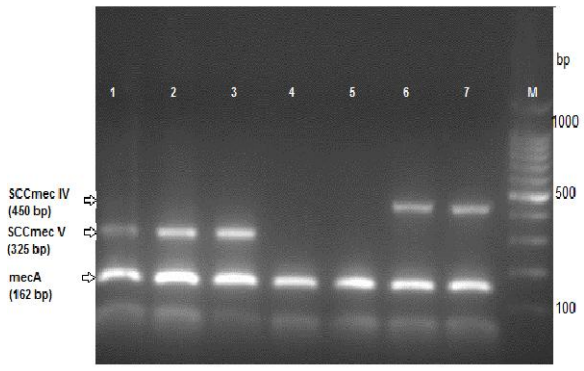

Figure 1: PCR assay for SCC*mec* typing and *mec*A gene detection. Lane1; positive control for Sccmec V, Lane 2 and 3; 325-bp Sccmec V gene fragment, Lane 4 and 5; negative Sccmec genes, Lanes 6; positive control for Sccmec IV gene, Lanes 7; 450-bp Sccmec IV gene fragment; all lanes show 162-bp *mec*A fragment; Lane M; 100-bp DNA ladder.

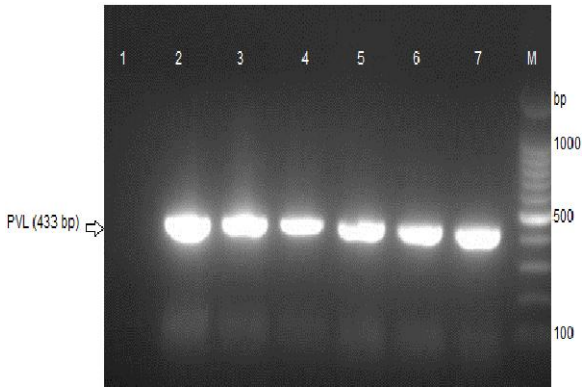

Figure 2: PCR detection of PVL gene among selected MRSA isolates. Lane 1; negative control; Lane 2; positive control, Lanes 3, 4, 5, 6 and 7; 433-bp PVL gene fragment, Lane M; 100-bp DNA ladder.

DISCUSSION

In Saudi Arabia, a tendency towards an increased number and severity of MRSA infections has been observed in the last decade. Many institutions have reported an increase in the incidence of MRSA [2, 3]. Some studies reported prevalence of MRSA in Jeddah [17, 18]. Mass gathering of individuals from all parts of the world in Makkah area increases their susceptibility to infections. Millions of pilgrims travel each year to perform Umrah and Hajj rituals, one of the most common infections those caused by MRSA infections [2, 3].

In this study, only 48.5% of bacterial isolates were confirmed as MRSA based on the presence of *mec*A gene which is responsible for mediating methicillin resistance in staphylococci [4]. The *mec*A gene is carried on the SCC*mec*. SCC*mec* is inserted into the *S. aureus* chromosome near the origin of replication [19]. The presence of the *mec*A gene correlated 100% with the methicillin phenotypic resistance in all MRSA isolates [20]. The emergence of methicillin-resistant staphylococcal lineages is due to the acquisition and insertion of the SCC*mec* element into the chromosome of susceptible strains. SCC*mec* typing is one of the most important molecular tools available for understanding the epidemiology and strain relatedness of MRSA [16]. CA-MRSA is frequently isolated from healthy people and tends to lead to serious and fatal outcomes. The main feature in this study, is the high number of MRSA that carry SCCmec type IV & V (44%) of which 30% were type IV and 14% were type V, which are traditionally attributed to CA-MRSA strains [21]. These results are in agreement with *Asghar* 2014 who reported that MRSA strains were increasing in both hospital and community settings in Makkah [22]. These data also is near international studies that reported the spread of CA-MRSA SCC*mec* type IV strains in hospital settings in both Europe and the United States [21]. In Japan, about 4% of MRSA clones are classified as SCC*mec* type IV [23, 24]. Another classical feature of CA-MRSA is the production of PVL. *Moussa and Shibl* in 2009 studied the genotypes of MRSA from Riyadh and found that CA-MRSA strains harbored the SCC*mec* type IV element and the PVL genes [25]. In this study the number of PVL positive strains was (21%) which is near to *Asghar* 2014, who reported (19%) in Makkah [22], and higher to the ones reported from European countries (3 to 15%) [23, 26]. Some studies from Geneva recorded high proportions (35%) of PVL-positive strains (2002 to 2005) [27]. In this study, PVL was strongly associated with SCC*mec* type IV (52.5%) and, to a lesser extent, type V (9.5%). The presence of CA-MRSA together with HA-MRSA in hospitals and occurrence of PVL-positive MRSA strains are indicators of the spreading of CA-MRSA from community to the hospital settings. This is because the CA-MRSA strains carrying SCC*mec* type IV or V have a selective advantage and able to grow faster and achieve highly infectious burdens than HA-MRSA strains [28, 29]. Due to the fastness and quality of molecular methods, they are strongly recommended for diagnosis and typing of MRSA pathogens. Additional infection control measures need to be considered to limit the spread of CA-MRSA in the holy places at Makkah especially during Hajj and Umrah seasons.

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