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Panton-Valentine Leukocidin and Staphylococcal Cassette Chromosome (SSC*mec*) from CA-MRSA (Community-Acquired Methicillin Resistanct *Staphylococcus aureus*).

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Abstract

Stapylococcal cassette chromosome mec (SSC*mec*) and Panton-Valentine leukocidin (PVL) gene were investigated among presumptative community-associated methicillin-resistant *Staphylococcus aureus* MRSA (CA-MRSA). CA-MRSA isolates carried the SCC*mec* from three samples out of healthy 100 college students as well as one sample of PVL positive. The risk of CA-MRSA, PVL positive and SSC*mec* was studied using a multiplex PCR.

Keywords: Stapylococcal cassette chromosome *mec* (SSC*mec*), Panton-Valentine leukocidin (PVL), community-associated methicillin-resistant *Staphylococcus aureus* MRSA (CA-MRSA)

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) becomes a primary cause of both health-associated (HA-MRSA) and community-associated (CA-MRSA) infections. The feature of MRSA is the Staphylococcal cassette chromosome *mec* (SCC*mec*).

SCC*mec* types are defined by the combination of the type of *ccr* (cassette chromosome recombinases) gene complex and the class of the *mec* gene complex. HA-MRSA strains tend to carry SCC*mec* types I, II, and III whereas SCC*mec* types IV and V elements are generally carried by CA-MRSA strain [1,2]. The resistance of *Staphylococcus aureus* to beta-lactam antibiotics is associated with an expression of penicillin-binding protein 2a (PBP2a) [3]. This protein is encoded by the *mec*A gene, which is situated on a mobile genetic element, sta

phylococcal cassette chromosome *mec* (SCC*mec*). Five different SCC*mec* types have been identified in methicillin-resistant *S. aureus* (MRSA) strains. SCC*mec* types I, II and III are mainly found in hospital-acquired [1,4]. MSRA (HA-MRSA), whereas SCC*mec* types IV and V are mainly associated with community-acquired MRSA(CA-MRSA).

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains producing the potent tissue necrotizing toxin Panton-Valentine leukocidin (PVL) encoded by the *pvl* gene, and harboring *SCCmec* type IV or V elements, have been implicated as being associated with community-acquired MRSA(CA-MRSA) infection [1,5]. CA-MRSA isolates carried the SCC*mec* type IV complex, and most were PVL positive [4]. In this study, we investigated the risk of *mec*A, PVL gene as well as SCC*mec* from CA-MRSA isolates of college students.

Materials and Methods

We chose 100 college students (*The ethical committee permission was taken for conducting this study, and all study participants were provided with informed and written consent) for this study and collected the presumptive samples from their nasal cavity and hands, respectively. Using a sterilized cotton swab, the isolates are obtained and transferred into Brain heart infusion (BHI) agar and MacConkey (Becton, Dikinson and Company, USA) for enrichment and selection, respectively. Followed by incubation for 24 hrs, we performed Gram stain. VITEK (bioMerieux, Maryl'Etoile, France)-automated system to identify bacteria. As a standard bacteria, *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27858) were used. We adapted the multiplex PCR assay previously published paper [6] and chose the lukS/F-PV genes (which encode the PVL S/F bicomponent proteins) with primers Luk-PV-1 (5'-ATCATTAGGTAAAATGTCTGGACATG ATCCA-3') and Luk-PV-2 (5'-GCATCAAGTGTATTGG ATAGCAAAAGC-3') [7], and the *mecA* gene (a determinant of methicillin resistance) with primers MecA1(5'-GTAGAAATGACTGAACGTCCGATAA-3') and MecA2 (5'-CCAATTCCACATTGTTTCGGTCTAA-3') [8].

The optimized multiplex PCR conditions were performed with the thermocycling conditions set at 94°C for 10 min, followed by 10 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 75 s and 25 cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 75 s. Four primer sets were designed to ensure amplification of two DNA targets from SCC*mec* type IV and two targets from SCC*mec* type V because it was reported that SCC*mec* types IV and V elements are generally carried by CA-MRSA strain [2,9]. Four primer sets [9] are GCCACTCATAACATATGGAA as 1272F1 and CATCCGAGTGAAACCCAAA as 1272R1 for SCC*mec* type I and IV, whereas TATACCAAACCC GACAACTAC as 5R *mec*A and CGGCTACAGTCA TAACATCC as 5R431 for SCC*mec*

type V. PCR amplification comprised 4 min at 94C, followed by 30 cycles of 30 s at 94C, 30 s at 55C and 60 s at 72C, with a final extension for 4 min at 72C. The SCC*mec* was determined on the basis of the band pattern obtained.

Results

Among 100 samples, 10 out of 15 samples of *S. aureus* were collected which are all over 90% VITEK results and resistant to oxacillin from antibiotic susceptibility test (not shown here).

From 10 *S. aureus* isolates, using multiplex PCR with *mec*A gene primer and PVL gene primer, 3 MRSA samples (lanes 2, 4, 8) are found as *mec*A (+). The frequency of MRSA from CA-MRSA of college students is only 3% (3/100) but no positive PVL gene expression from MRSA isolates (Figure 1). However, one PVL positive gene (lane 5) is detected from MSSA, not MRSA samples.

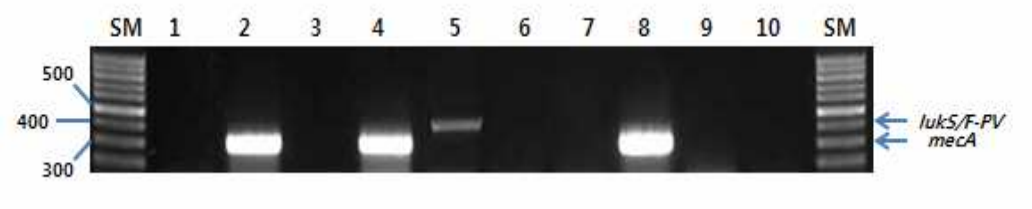

Figure 1. *Detection of PCR products of mecA and LuK-PV of MRSA isolates*
Lane 1~10 are S. aureus; Lane 2,4,8 are mecA gene expression (MRSA); Lane 5 indicates Panton-Valentine Leukocidin (PVL) from MSSA.

Primers, 1272F1/1272R1 for SCC*mec* were designed for SCC*mec* type I and IV, while 5RmecA/5R431 for SCC*mec* type V [9]. With same 10 samples, we performed the detection of SCC*mec* genes. Lane 2, 8 and 9 indicates SCC*mec* I or IV (can't decided here), whereas lane 4 shows SCC*mec* type V (Figure 2). Sample 9 was not shown as a MRSA (Figure 1), but can be carried with SSC*mec* (Figure 2), This can be expected that MSSA also can carry with SSC*mec*

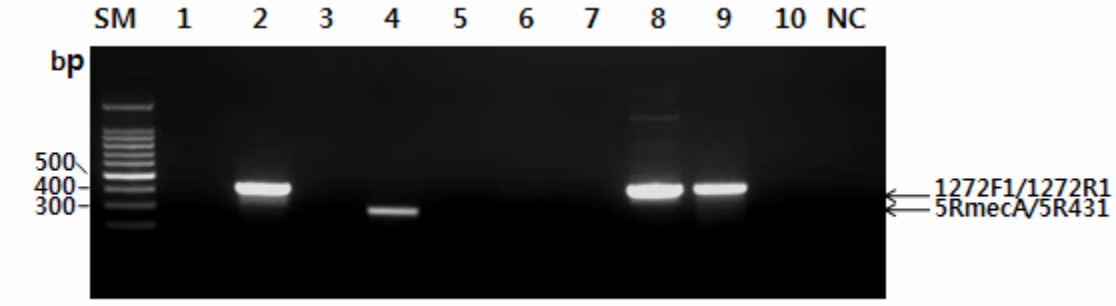

Figure 2. SCCmec type from S. aureus isolates
Lane 2,8 and 9 indicate SCCmec I or IV, whereas Lane 4 shows SCCmec type V
**Abbreviation: SM-size marker, NC-negative control*

Discussion

Panton-Valentine leukocidin is a biocomponent leukocidin encoded by two cotranscribed genes, lukS-PV and lukF-PV (lukS/F-PV), which cause leukocyte destruction and tissue necrosis [6]. PVL is also an *S. aureus*-specific exotoxin and its genes have been demonstrated primarily among CA-MRSA [4].

Several studies shown that PVL genes were found <5% of *S. aureus* isolates worldwide [7,10,11], whereas our study was shown 1% PVL positive from CA-MRSA. However, the rates of carriage of the PVL toxin gene are >75% for CA-MRSA stains [12] and 67% and 80% for CA-MRSA strains with ST8 and ST1, respectively, in the United State [13]. This big different rate of PVL positive from

CA-MRSA might be caused between bacterial strains and direct isolates from health human skin of this study. Consequently, the reported articles were studied with MRSA bacterial strains, which are presumptively carrying PVL gene. It is expected that CA-MRSA infection is associated with community PVL-positive MRSA nasal and skin carriage but not detected from CA-MRSA but only one PVL-positive MSSA was shown.

For the detection of PVL and methicillin resistance (*mec*A) genes represents a new tool to aid the early identification of CA-MRSA isolates [6]. Using multiplex PCR, both of two parameters, PVL and *mec*A did not find from our 10 samples but separately detected. The identification of *S. aureus* isolates carrying PVL genes is an important first step in controlling the virulence [6]. The combination of both PVL and *mec*A shows very potent virulence with resistant to antibiotics such as oxacillin. Consequently, it is very important to identify serious and harmful *S. aureus* from both detection of PVL and mecA. However, several studies are shown such as PVL(-) MSSA, PVL(+) MSSA, PVL (-) MRSA and PVL (+) MRSA.

The *pvl* genes are more common in CA-MRSA isolates compared with CA-MSSA isolates [14,15,16] and a recent molecular study showed the presence of *pv*l in almost all of the CA-MRSA strains [17].

From the multiplex PCR for *mec*A and PVL gene, three *mec*A genes and one PVL gene from lane 2, 4, 8 and lane 5, respectively. However, it was expected that *mec*A and PVL genes are simultaneous expression but not correlated. One PVL gene is expressed in lane 5 only, which is MSSA. The PVL toxin can be carried by both MRSA (meticillin resistant *Staphylococcus aureus*) and MSSA (meticillin sensitive *Staphylococcus aureus*).

The resistance of bacteria is caused by the acquisition of the methicillin resistance gene *mec*A, carried by the staphyloccal cassette chromosome *mec* (SCC*mec*). By the time the Guidelines for the classification of SCC*mec* elements were prepared, eight SCC*mec* types have been described for *S. aureus* [18]

SCC*mec* types IV and V elements are generally carried by CA-MRSA strain was reported [1,2]. Therefore, it is expected that samples 2,4,7 and 8 are carrying *mec*A gene from CA-MRSA but sample 7 did not show *mec*A gene from Figure 1 because the feature of MRSA is the Staphylococcal cassette chromosome *mec* (SCC*mec*). However, CA-MRSA isolates carried the SCC*mec* type IV complex, and most were PVL positive, whereas the HA-MRSA isolates carried the SCC*mec* type II complex and did not habor the PVL genes [4,19].

As a result of Figure 2, lane 2,8 and 9 shows SCC*mec* I or IV and lane 4 indicates SCC*mec* V.

Sample 9 was not shown as a MRSA (Figure 1), but can be carried with SSC*mec* (Figure 2), This can be expected that MSSA also can carry with SSC*mec*. CA-MRSA generally carries *mec*A gene with PVL-positive and SCC*mec*. It is found that three samples, lane 2, 4 and 8 can be reached these two parameters, *mec*A and SCC*mec*. There is no sample with three criteria, *mec*A, PVL-positive and SCC*mec* from CA-MRSA isolated. This rates only 3% with both *mec*A and SCC*mec* because 100 college health students' samples were used.

PVL is a surely virulent factor for skin necrosis, pulmonary lung infections as well and MRSA is becoming a serious bacteria in the worldwide with resistant to various antibiotics.

In this study, it is performed from 100 health college students, showing not high rate of PVL-positive *mec*A and SCC*mec*. However, it can be detected from even health human skin or nasal. This will be provided that the higher rate on the occurrence of three parameters can be happened on the exposure of poor environments through community-acquired route.

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