Panton-Valentine Leukocidin and Staphylococcal Cassette Chromosome (SSCmec) from CA-MRSA (Community-Acquired Methicillin Resistant Staphylococcus aureus).

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Abstract

Staphylococcal cassette chromosome mec (SSCmec) and Panton-Valentine leukocidin (PVL) gene were investigated among presumptive community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA). CA-MRSA isolates carried the SSCmec 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 SSCmec was studied using a multiplex PCR.

Keywords: Staphylococcal cassette chromosome mec (SSCmec), 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 (SCCmec).

SCCmec 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 SCCmec types I, II, and III whereas SCCmec 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 mecA gene, which is situated on a mobile genetic element, sta

Staphylococcal cassette chromosome mec (SCCmec). Five different SCCmec types have been identified in methicillin-resistant S. aureus (MRSA) strains. SCCmec types I, II and III are mainly found in hospital-acquired [1,4]. MSRA (HA-MRSA), whereas SCCmec 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 SCCmec type IV complex, and most were PVL positive [4]. In this study, we investigated the risk of mecA, PVL gene as well as SCCmec 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, Dickinson 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’-ATCAATTAGTAAAAAGGCTCAGATG ATCCA-3’) and Luk-PV-2 (5’-CATCAAAGGTATTTGG ATAGCATAAG-3’) [7], and the mecA gene (a determinant of methicillin resistance) with primers MecA1(5’-GTAGAAATGACTGAACGTCCTGATAA-3’) and MecA2 (5’-CCAATTCCACATTGTTCCGTCTAA-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 SCCmec type IV and two targets from SCCmec type V because it was reported that SCCmec types IV and V elements are generally carried by CA-MRSA strain [2,9]. Four primer sets [9] are GCCACTCATACATATGGAA as 1272F1 and CATCCGAGTGAACCCAAA as 1272R1 for SCCmec type I and IV, whereas TATACAAAAACCCGACAACT as 5R mecA and CGGCTACAGTCA TAAACATCC as 5R431 for SCCmec type V. PCR amplification comprised 4 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C and 60 s at 72°C, with a final extension for 4 min at 72°C. The SCCmec 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 mecA gene primer and PVL gene primer, 3 MRSA samples (lanes 2, 4, 8) are found as mecA (+). 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.

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 strains [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 (mecA) genes represents a new tool to aid the early identification of CA-MRSA isolates [6]. Using multiplex PCR, both of two parameters, PVL and mecA did not find from our 10 samples but separately detected. The identification of Staphylococcus aureus isolates carrying PVL genes is an important first step in controlling the virulence [6]. The combination of both PVL and mecA 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 pvl in almost all of the CA-MRSA strains [17].

From the multiplex PCR for mecA and PVL gene, three mecA genes and one PVL gene from lane 2, 4, 8 and lane 5, respectively. However, it was expected that mecA 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 meticillin resistance gene mecA, carried by the staphylococal 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 mecA gene from CA-MRSA but sample 7 did not show mecA 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 harbor 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 SCC mec (Figure 2). This can be expected that MSSA also can carry with SCC mec. CA-MRSA generally carries mecA gene with PVL-positive and SCC mec. It is found that three samples, lane 2, 4 and 8 can be reached these two parameters, mecA and SCC mec. There is no sample with three criteria, mecA, PVL-positive and SCC mec from CA-MRSA isolated. This rates only 3% with both mecA 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 mecA 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.

References


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