ORIGINAL ARTICLE

Panton-valentine leukocidin, *mecA*, and *SCCmecV* in methicillin-resistant *Staphylococcus aureus* isolates sampled from hospitalized patients in Northern Iran

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Abstract

Introduction: Panton-Valentine Leukocidin (*pvl*) is associated with strains of *Staphylococcus aureus* that produce a high level of virulence. The aim of this study was to investigate the prevalence of *pvl* gene and its relationship with *mecA* and *SCCmecV* in isolated samples taken from hospitalized patients in northern Iran. **Materials and Methods:** During a 6-month treatment period, a total of 92 clinical isolates of *S. aureus* were obtained. Resistance to methicillin was determined, and the prevalence of *pvl* gene was estimated through running a polymerase chain reaction (PCR) on chromosomal DNA. The *pvl* positive isolates were further analyzed for *mecA* and *SCCmecV* genes by PCR. **Results:** In total, 18 isolates (19.56%) were shown to be positive in terms of their carrying the *pvl* gene, and from among them, 15 isolates were methicillin-resistant *S. aureus* (MRSA), 3 were methicillin-susceptible *S. aureus*, and 8 were positive for *mecA* gene. None of the *pvl* positive samples had the *SCCmecV* gene cassette. **Conclusions:** The study found that the majority of *pvl* positive isolates were MRSA, and almost half of them had the *mecA* gene. Based on the results, it could be postulated that there is a significant relationship between these two variables; however, they were not correlated in regard to *SCCmecV*.

Key words: *mecA*, methicillin, methicillin-resistant *Staphylococcus aureus*, Panton-Valentine Leukocidin, *SCCmec*

INTRODUCTION

Staphylococcus aureus a Gram-positive cocci is similar in appearance to a bunch of grapes under a microscope and produces relatively big yellow colonies on culture medium. It is often isolated from the nose, mouth, and digestive tract. It is also responsible for most hospital-acquired infections as well as intravascular infection.[1] It is often isolated from cases of pneumonia, arthritis, endocarditis, osteomyelitis, and septicemia infections. Pathogenicity of *S. aureus* relates to a product of compounds, including viscous matrix molecules (such as mass maker factor), extracellular proteins (such as coagulase and hemolysin), enterotoxins, exfoliative toxins toxic shock syndrome toxins, and Panton-Valentine Leukocidin (*pvl*).[2] Panton-Valentine is one of the most important factors of virulence isolated by Panton and Valentine from a patient with furunculosis in 1932. The toxin consists of two protein parts of S (38 KDa) and F (32 KDa) controlled by *LukS/pv* and *Lukf/pv*, respectively. Its main target is the extracellular membrane of polymorphonuclear, monocytes, and macrophages. Depending on the level of toxin concentration, it leads to the opening of calcium channels as well as of necrosis and apoptosis in leukocytes.[3,4] *pvl* is associated with methicillin-resistant *S. aureus* (MRSA) strains; however, *LukS/LukF-PV* genes can be carried by strains of methicillin-susceptible *S. aureus* (MSSA) as well.[4] Strains of the *pvl*

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positive *S. aureus* produce a high level of virulence which is often associated with skin abscesses and acute necrotic infection.[3] The genes for MRSA and *SCCmec* are SCC chromosomal cassettes of a relatively large DNA fragment, which are integrated into the *orf x*gene within *S. aureus* chromosome. SCCs can code resistance to antibiotics and/or agents of virulence determinants. Considering that SCCs can be divided into *SCCmec* and non *SCCmec* groups, all MRSA strains contain *SCCmec*, which codes for the *mecA* genes. Methicillin-resistant strains produce a new type of penicillin-binding protein (PBP) known as PBP2, which has a low affinity with methicillin or any other β-lactam antibiotics. Accordingly, peptidoglycan synthesis continues inside the cell wall of bacteria despite the presence of these drugs.[5,6] The *mecA* gene and its regulatory elements comprise the *mec* complex altogether. Until today, 11 SCCmec types have been identified that can be differentiated from each other by their carrying the *ccr* gene complex. In terms of size, *SCCmecI*, *SCCmec II,* and *SCCmecIII* are larger, and *SCCmecIV* and *SCCmecV* are smaller.[7,8] In recent years, numerous studies have shown an increase in the variety of methicillin resistance and of *pvl* positive *S. aureus*. Considering its importance, the current study, then, sought to investigate the prevalence of the *pvl* gene and its relationship with *mecA* and *SCCmecV* in strains isolated from hospitalized patients in Northern Iran.

MATERIALS AND METHODS

Bacterial isolates

From February to July of 2015, a total of 92 clinical *S. aureus* strains were isolated from wounds, blood, urine, splinter, and body fluids of hospitalized patients in Rasht, Northern Iran. Clinical specimens were initially inoculated in Mannitol Salt Agar and incubated at 37°C for 18–24 h. Colonies were examined for catalase production, coagulase, hemolysin, DNase, as well as mannitol fermentation.[9]

Susceptibility testing and polymerase chain reaction (PCR)

An antibiotic susceptibility test was carried out by capitalizing on the disk diffusion method based on Clinical and Laboratory Standards Institute (CLSI) 2015 protocols. The antibiotic disks that were tested involved vancomycin (30 µg), oxacillin (1 µg), teicoplanin (30 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), cefoxitin (30 µg), and linezolid (10 µg). In addition, a DNA extraction kit) Roche High Pure PCR Template Preparation Kit, Germany) was used to extract DNA from bacterial colonies.[10] In this study, *S. aureus* (ATCC33591-*mecA gene*), *S. aureus* (ATCC49775-*pvl gene*), and *Staphylococcus epidermidis* (ATCC12228) considered as a methicillin resistance of positive, positive and negative controls, respectively. Using specific primers for the *pvl* gene*,* a PCR was used for detection of this gene in the genomic DNA of all *S. aureus* isolates. Those samples which were found to be positive for the *pvl* gene were subjected to additional PCR assays in an attempt to search for *mecA*, *LukS- PV-F, mecA,* and *SCCmecV* genes. The sequences of all primers used are summarized in Table 1. Primers were evaluated by the oligo analysis online software and NCBI base.[9] The collected data were analyzed by the help of a Chi-square test and using SPSS-v16.

PCR was performed in a final 25 µl volume reaction containing PCR buffer (×10), magnesium chloride (2 mM), deoxynucleotide triphosphates (0.2 mM), forward and reverse primers (10 pmol/µl), template DNA (1 µl), Taq DNA polymerase (1.5 µl), and deionized water. The PCR method was performed in a thermal cycler (Bio-Rad, USA) according to the following program: Initial denaturation (95°C, 30 s), 32 cycles each composed of initial denaturation (95°C, 30 s), primer annealing (62°C, 30 s), and extension (72°C,60 s).[11,12] Positive control for *mecA*, *pvl, SCCmec V* genes, and negative control (distilled water) was also regarded in each series of the PCR reaction. The PCR product was subjected to electrophoresis in 1% agarose gel containing DNA safe stain and was documented using gel documentation (UVI TEC, Cambridge, UK).[11,12]

RESULTS

A total of 92 *S. aureus* were recovered from hospitalized patients in Rasht, Northern Iran. They were obtained from 20 urine specimens, 39 wounds, 17 blood samples, 9 chips, and 7 body fluid samples [Figure 1]. The antibiotic susceptibilities of these isolates are illustrated in Table 2.

As can be seen, the PCR assays suggest that 18 isolates (19.56%) were positive in terms of the existence of *pvl* gene, and from among these, 15 isolates (83.33%) were MRSA, 3 (16.66%) were MSSA, and 8 (8.69%) were in possession of *mecA*. Out of the 18 *pvl*- positive isolates, 9 were obtained

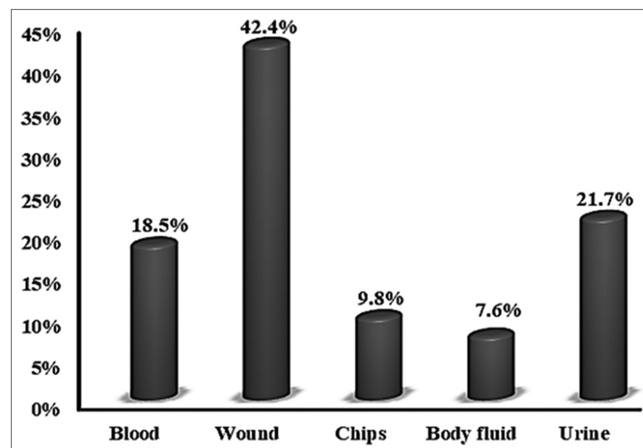

Figure 1: Number of *Staphylococcus aureus* isolated obtained from different sources (92)

Table 1: The sequences of primer pairs used in the PCR assays

Primer	Sequence (5'–3')	Size	Reference
mecA- F mecA- R	GTGAAGATATACCAAGTGATT ATGCGCTATAGATTGATTGAAAGGA	146bp	[13]
Luk *pvl* - F Luk *pvl* - R	TCATTAGGTAAAATGTCTGGACATGATCCA GGATCAAGTGTATTGGATAGCAAAAGC	433bp	[13]
SCCmec V- F SCCmec V- R	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	325bp	[14]

PCR: Polymerase chain reaction

Table 2: Susceptibility of *S. aureus* isolates to antibiotics

Antibiotics	Number (%)		
	Resistant	Intermediate	Sensitive
Vancomycin	2 (2.17)	30 (32.6)	60 (65.21)
Oxacillin	43 (46.4)	25 (27.17)	25 (27.17)
Teicoplanin	12 (13.04)	36 (39.13)	44 (47.82)
Gentamicin	22 (23.91)	31 (33.69)	39 (42.39)
Tetracycline	21 (22.82)	45 (48.91)	26 (28.26)
Ciprofloxacin	28 (30.43)	16 (17.39)	48 (52.17)
Cefoxitin	14 (15.21)	45 (48.91)	33 (35.86)
Linezolid	26 (28.26)	29 (31.52)	37 (40.21)

S. aureus: Staphylococcus aureus

from specimens, 3 from urine, 3 from body fluids, 2 from blood, and 1 from splinter. None of *pvl*- positive samples had the gene cassette. The PCR amplification of *mecA* and *pvl* genes has been shown in Figure 2. The results were analyzed by Fisher test with 95% confidence. The results revealed that there is no linear correlation among *pvl, mecA,* and *SCCmecV* genes (./8% Cramer test).

DISCUSSION

Antibiotic resistance has proved to be a growing problem in medical settings. The problem is especially menacing in regard to *Staphylococci* due to its inherent higher pharmaceutical resistance than other strains of bacteria. *pvl* is a virulence agent carried by a bacteriophage and can be transferred to other staphylococci.[6,13] Strains of *pvl* positive *S. aureus* have a high level of virulence and are responsible for acute infections, including brain, bone and joint infections, and necrotizing pneumonia.[14] Therefore, prompt diagnosis and eradication of *pvl* producing are of paramount importance. In a study conducted by Orrett and Land, of 243 strains of the *S. aureus* isolated from the hospital sources, 20.8% of them are MRSA.[15] Cupane *et al.,* 2015, reported that 75.0% of this *S. aureus* isolates possessed the *pvl* gene. Brown *et al*., 2012, checked 1055 isolates of *S. aureus* for *LukSF/PV* genes. In their experiment, 377 of these isolates (35.7%) were found to be positive for it. Miller *et al*., 2007, reported that 108 of their total 180 *S. aureus* isolates were MRSA.

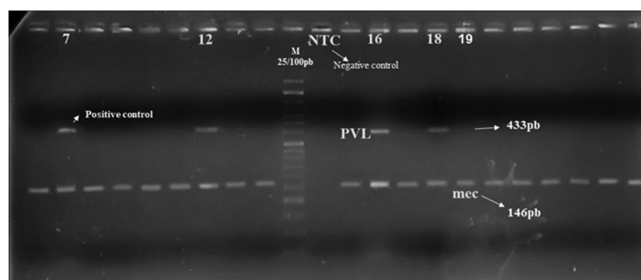

Figure 2: Agarose gel electrophoresis of polymerase chain reaction product for detection of the Panton-Valentine Leukocidin (*pvl*) and mecA gene, lanes 12, 16, 18 positives *pvl* samples, and 19 positive mecA sample. NTC: Negative control, lanes 7: Positive control, ladder 25/100 bp

Molla-abaszadeh *et al*., 2013, reported that of their 100 *S. aureus* isolates, 18 (18.0%) were carriers of the *pvl* gene, and 94.4% of these were MRSA, and 5.6% MSSA. Darbi *et al*., 2012, who recovered *S. aureus* isolates from patients and hospital employers, reported rates of resistance to methicillin to be 90.0%. In line with the findings of the present study, most of *pvl* positive strains have been (up to 89.0%) MRSA, and only 11.0%of them have been MSSA. Its well-known that due to unnecessary consumption of antibiotics, genetics exchanges specifically resistance genes occurs through plasmids. Therefore, a partial resistance of *S. aureus* infectious treatment could be performed. In addition, ample evidence revealed that in 30–50% of *S. aureus* methicillin resistance process will take place by *mecA* gene.[16] Of these isolates, 18 were harbored *pvl* genes all of which were collected from skin and soft tissue infections. In the present study, of 18 *pvl* positive isolates, 10 were isolated from wounds. In another study, of 119 isolates of *S. aureus* that were recovered from outpatients, 67.4% were MRSA. In 2014, of 131 MRSA isolates, which had been isolated from the nose and throat of healthy individuals in Mexico city, 21.4% were shown to be MRSA. Of them, 2.3% were harbored *SCCmec V*; however, this gene was not detected in this study.[17] In a study conducted, in 2011, in Tehran, Iran, 7 MRSA isolates were identified in 154 nostril isolates of *S. aureus*. In this study, only one isolate had the *pvl* gene, and of 7 MRSA isolates, only one was positive for *SCCmecV*.[18] Of 202 *S. aureus* isolates collected in Chinese hospitals, *SCCmec* and *pvl* gene were observed in 10 isolates, and three of these were harbored *SCCmecV*.[19] In this study, of 93 isolates of *S. aureus*, 44 (47.82%) were found to be MRSA. 18 isolates

(19.56%) were positive in terms of the *pvl* gene, and among these, 83.33% were MRSA, and three (16.66%) were MSSA. Eight cases (8.69%) were positive for *mecA*, and none of the *pvl*-positive samples had the *SCCmecV* gene cassette. In a study conducted in 2015, 30% of the total 200 *S. aureus* isolates had *mecA* gene in which 6% contained *pvl* gene, and interestingly, none of the *mecA* positive isolates were *pvl* positive.[20] Related to the existence of PBP2a protein, it is codified by the *mecA* gene and has a low compound tendency to β-lactams. It is placed in the chromosomal staphylococcal (SCCmec) cassette of the *mecA* gene.[21]

On the basis of these findings, sensitivity or resistance pattern in *S. aureus* to routine antibiotics treatment is different around the world, therefore, the control of antibiotics medication should have a critical role in resistance inhibition process. It should be noticed that production of toxins through *S. aureus* is an important issue in individual health, so, using an appropriate laboratory method should be considered to the recognition of infectious factor that could be beneficial strategy for rapid diagnosis in other species and toxins.

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