The Prevalence of Panton-Valentine Lukocidin Gene in *Staphylococcus aureus* Species Isolated From Nosocomial Infections in Isfahan, Iran

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**Abstract**

**Background:** *Staphylococcus aureus* is a significant pathogen and major cause of nosocomial and community-acquired infections. The current study aimed at investigating the frequency of Panton-Valentine leukocidin (PVL) gene as a serious virulence factor causing WBC destruction.

**Methods:** Collectively, 100 species of *S. aureus* were isolated from Isfahan, Iran, hospitals and confirmed by biochemical tests (coagulase, mannitol fermentation, and DNase). The antibiotic resistance patterns were studied by the disk diffusion method.

**Results:** Out of the 100 isolates, 56.2% were **PVL** positive of which 19.8% from abscess, 51.7% from wound, 23.2% from bedsore, and 5.3% from tracheal secretion. Among the detected isolates, 87.8% were resistant to methicillin.

**Conclusions:** The current study showed the high frequency of **PVL** in wound strains. Further studies are required to understand the distribution of these virulent isolates in order to decrease the risk of infection. High quality hospital cares as well as new antibiotics is required to combat the multidrug resistant bacteria.

**Keywords:** Panton-Valentine Leukocidin, Nosocomial Infections, *Staphylococcus aureus*

1. **Background**

   Increased bacterial resistance to antibacterial agents due to indiscriminate use may result in an inexpressive array of substances to battle some bacterial infections; it shows the importance of antibiotic resistance pattern in *Staphylococcus aureus* (*S. aureus*) known for a long time as a principal pathogen of hospital-acquired infections (1).

   One of the most important virulence factors of these bacteria is leucocidin (**PVL**), which are toxins with 2 separate synergic conformations. Infections caused by these bacteria are mainly controlled with antibiotics such as methicillin or aminoglycosides, which their resistance pattern changes every day. Methicillin is the first-line treatment and resistant to it in *Staphylococcus aureus* is mediated by a penicillin binding protein (PBP2A) encoded by the *mecA* gene (2).

   Previous studies demonstrated that *S. aureus* species are the main reason for skin and soft tissue infections such as impetigo, furunculosis, bedsores, surface and surgical wounds, and abscess, and further systemic infections such as pneumonia, urinary tract infections (UTIs), and endocarditis (3).

   It is suggested that panton-valentine leukocidin (**PVL**), as a significant virulence factor, was for the first time identified by Panton and Valentine from a supernatant suspension of *S. aureus* V8 isolated from a patient with chronic furunculosis infection. The important virulence factors most relevant to community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in many infections including pneumonia, wound infections, conjunctivitis or folliculitis are the collagen-adhesion proteins, super antigens, and the pore-forming toxins, specifically the **PVL** and alpha-hemolysis. Moreover, phenol-soluble modulins are produced especially in high amounts in CA-MRSA strains, whereas the production is lower in typical hospital-acquired (HA)-MRSA; but recent studies showed enough connection between community- and hospital-acquired infections (4).

   The **PVL** includes S and F proteins, ingredients that operate interdependently and none acts by itself. These dimeric molecules are connected to each other and assemble in human polymorph nuclear cells membrane to form an octameric structure and open Ca²⁺ channels. Studies found that the antibiotic resistance pattern, the frequency of the **PVL** gene, and the determination of different types
of isolates, based on the hospital section and type of infection, were the main purposes considered in the current study. The current study aimed at determining the frequency of PVL gene in S. aureus isolated from hospital infections in Isfahan, Iran (5).

2. Methods

2.1. Bacterial Isolates

Samples were collected and S. aureus was identification in 6 months at 3 hospitals of Isfahan; 100 clinical samples from various infections were collected and 100 S. aureus species including bed sore wound (n = 23.2%), wound (n = 51.7%), abscess (19.8%), tracheal secretion (5.3%) were identified. All samples were immediately cultured on 7% sheep blood agar (Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 48 hours. After incubation, suspected colonies were tested by microbiological techniques to diagnose Staphylococcus spp. (6).

According to other studies, the API-20 Staph system kit (bio Merieux, France) was also used for the final verification. The grown colonies were tested for S. aureus based on colony characteristics, Gram staining, pigment production, as well as the following biochemical reactions: catalysis activity, coagulated test (rabbit plasma), oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on mannitol salt agar (MSA) (Merck, Darmstadt, Germany), nitrate reduction, urease activity, phosphatase, deoxyribonuclease (DNase) test, novobiocin resistance, and carbohydrate fermentation tests. Five MRSA strains, NCTC10442, N315, 85/2082, CA05, and WIS (WGB8318), were used as the standard strains (7).

2.2. Antibiotic Susceptibility Testing

Staphylococcus aureus isolates were selected and then, the antibiotic resistance pattern was investigated by the disc diffusion method (on Mueller-Hinton agar). Staphylococcus aureus isolates were tested for susceptibility to penicillin (10 U), imipenem (10 µg), cefazolin (30 µg), ceftaxin (50 µg), gentamicin (10 µg), ciprofloxacin (5 µg), clindamycin (2 µg), azithromycin (15 µg), erythromycin (15 µg), mupirocin (30 µg), rifampicin (5 µg), tetracycline (30 µg), trimethoprim (5 µg), vancomycin (30 µg), and nitrofurantoin (300 µg), by the Kirby-Bauer disk diffusion method (MAST, Merseyside, England), according to Clinical and Laboratory Standards Institute (CLSI) 2011. According to another studies, S. aureus ATCC 25923 was used as the control strain (8). For DNA extraction and Staphylococcus confirmation, a generic colony of the biochemically identified S. aureus was cultivated in 1 ml. trypticase soy broth (TSB) for 24 hours at 37°C, then the bacterial genomic DNA of S. aureus strains were exploited with a QIA-GEN plasmid Mini Kit (Fermentas, Germany) according to manufactures’ recommendation (9).

All the isolates were tested for PVL and mecA genes by the multiplex polymerase chain reaction (M-PCR) in which standard strain NCTC 13300 was the positive control, and distilled water was the negative control. Since methicillin-resistant isolates were the highest detected strains, molecular method was used for identification too. Finally, DNA was amplified on an Eppendorf thermal cycler in a final volume of 50 μL containing 5 μL of 10x buffer, 3 μL of MgCl2, and 1.5 μL of dNTP (10 pmol), 20 pmol of each primers (Luks-F/PVL-1 and Luks-F/Pvl-2), 32.5 μL of distilled water and 4 μL of the extracted DNA. Finally, the selected isolates were denatured for 5 minutes at 95°C following 35 cycles for denaturation for 30 seconds at 92°C, annealing for 30 seconds at 55°C, and extension for 45 seconds at 72°C. Eventually, final amplification was performed at 72°C for 10 minutes. PCR products were analyzed by electrophoresis through a 1.5% agarose gel. The primers used for M-PCR are shown in Table 1 (10, 11).

Statistical analysis was conducted with SPSS version 12.0 to analyze the relationship between the frequency of PVL and mecA harboring species and the patient’s age and gender. Finally, Chi-square test was used to determine the statistical significance. In the current study, P values ≤ 0.05 at 95% confidence interval were considered significant (2).

3. Results

In the current cross sectional study, 100 isolates of S. aureus were collected in 6 months. The isolates were collected from Al-Zahra, Kashani, and Shariati hospitals affiliated to Isfahan University of Medical Sciences. Based on the frequency of isolates detected from different departments of hospitals, orthopedics (%35.3) was the most infected department. The current study found a high prevalence of multi-drug resistant (MDR) S. aureus strains in hospitalized patients. Antibiotic resistance pattern of the studied isolates are shown in Table 2.

According to the result of the current study, out of 100 isolates, 56.2% were PVL positive of which 19.8% isolated from abscess, 51.7% from wound, 23.2% from bed sore, and 5.3% from tracheal secretion. Among the detected isolates, 87.8% were resistant to methicillin. Of the methicillin-resistant isolates, 80.2% harbored mecA gene; 69.2% were detected from females, and 30.8% from males. Due to increasing trend of drug resistance, further studies on larger sample sizes are necessary and can be useful to control hospital infections. The mean age of hospitalized patients...
Table 1. Genes Primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequences of Primer (5’ - 3’)</th>
<th>Amplicon Size, bp</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luk/PV</td>
<td>Luk PV1 5’ATCATTAGTAAAATGTCTGCACATGATCCAA 3’</td>
<td>433</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td>Luk PV-2 5’GCATCAASTGTATTGGATAGCCAAAAGC3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MecA</td>
<td>F: GTGAMAGTGACTGAAGCTCCGATAA</td>
<td>310</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>R: CCAATTCGACATTGTCGGTGCTAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Antibiotic Susceptibility Pattern of S. aureus Isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>13.1</td>
<td>8</td>
<td>78.9</td>
</tr>
<tr>
<td>Imipenem</td>
<td>17.8</td>
<td>23</td>
<td>59.2</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>24.3</td>
<td>30</td>
<td>45.7</td>
</tr>
<tr>
<td>Cefalotin</td>
<td>41.7</td>
<td>39</td>
<td>19.3</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>29.9</td>
<td>10</td>
<td>61</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>13.1</td>
<td>13.4</td>
<td>73.6</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>7</td>
<td>10</td>
<td>83</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>15.0</td>
<td>56</td>
<td>29</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>24.5</td>
<td>12.5</td>
<td>63</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>3.8</td>
<td>23</td>
<td>73.2</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>60.1</td>
<td>21.2</td>
<td>18.7</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>49.9</td>
<td>32</td>
<td>18.1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>19.5</td>
<td>34.5</td>
<td>46</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>59.0</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>90.8</td>
<td>7.2</td>
<td>2</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>81</td>
<td>7</td>
<td>12</td>
</tr>
</tbody>
</table>

*Values are expressed as No. (%).

that the isolates were taken from was 56 years, and according to the Student t test, there was no significant relationship between the age of patients and the presence of PVL gene, but there was a significant relationship between the detected genes and antibiotic resistance, especially resistance to methicillin.

4. Discussion

The results of the current study showed that 80.2% of all Staphylococcus strains harbored mecA coding resistance against methicillin. In addition to methicillin, the Staphylococcus strains showed resistant against some antibiotics such as macrolides, erythromycin, lincosamides, aminoglycosides, and tetracycline. Staphylococcus strains of the current investigation had the highest levels of antibiotic resistance against erythromycin (73.2%), ciprofloxacin (83%) and penicillin (78.9%). The lowest resistance rates were also against vancomycin (2%) and nitrofurantoin (12%).

The results of some other studies were in agreement with those of the current one, the frequency of PVL was 61% in a study by Alghaithy in Saudi Arabia; Moussa in Jeddah showed that the majority of PVL-harboring S. aureus (n = 18; 39.1%) was isolated from soft tissue and wound infections. Moreover, the PVL gene was detected in patients with pneumonia and respiratory infections (n = 7; 25.0%); while, Rijals reported 56.1% in Bokhara, Tajikistan. The most commonly used antibiotics included oxacillin, nalidixic, and vancomycin and S. aureus strains showed the highest resistance to these bacteria in different reports (13-45).

Of the 100 isolates, 56.2% were PVL positive of which 19.8% were isolated from abscess, 51.7% from wound, 23.2% bed sore, and 5.3% from tracheal secretion. Among all of detected isolates, 87.8% were resistance to methicillin. However, the prevalence of this gene is report 65% amongst S. aureus isolates. These differences in the prevalence rate maybe due to different geographical areas and the type of assay used to detect the gene. Another study detected PVL in S. aureus using the agar gel immunodiffusion (AGID) test in a hospital in France and reported that the PVL-producing S. aureus isolates were responsible mostly for necrotizing cutaneous infections such as furuncle and abscess (16, 17).

It is noted in several reports that a patient with abscess, revolving furuncle, or wound infections should be primarily examined for PVL-producing S. aureus (18). PVL-producing S. aureus is specifically detected in high-risk groups such as athletes. In the present study, Luk/PV, PVL, and mecA genes were detected using M-PCR and analyzed by electrophoresis on 1.5% gel agarose. The results were similar to those from other researches. The findings were in agreement with those from Wannet et al. in Holland (19). Generally, the results of the research showed the high prevalence of PVL-producing S. aureus in the hospitals under study. MRSAs are resistant to a wide range of antibiotics including methicillin; the result also reported in different studies (12).
5. Conclusions

It is hard to control the virulence strains of S. aureus resistant to various types of antibiotics. Infections with such strains emphasize the need for high quality medical cares as well as novel antibiotics. Hence, the pivotal role of clinicians is the judicious prescription of antibiotics. Physicians should thus take suitable strategies for the prognosis of such isolates as well as quick and proper therapeutic actions. It is therefore very important to identify and de-colonize the carriers because infections with such isolates are very invasive and even lethal and their epidemics may impose irreversible burden.

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Footnotes

Conflict of Interest: The authors declared no conflict of interest.

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