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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, bedsore, 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 Ca^{+2} 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 bedsore 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, hemolytic activity, 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), cefalotin (30 μg), ceftriaxone (30 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 other 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 QIAGEN 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 $MgCl_2$, and1.5 μL of dNTP (10 pmol), 20 pmol of each primers (Luks-F/*PVL*-1 and Luks-F/pv-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 propagation 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 conduct 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 bedsore, 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

Gene	Sequences of Primer (5' - 3')	Amplicon Size, bp	Reference
Luks/F-PV	Luk PV1 5'ATCATTAGGTAAAATGTCTGCACATGATCCA3'	433	(12)
	Luk PV-2 5'GCATCAASTGTATTGGATAGCCAAAAGC3		
MecA	F: GTAGAAATGACTGAACGTCCGATAA	310	20
	R: CCAATTCCACATTGTTTCGGTCTAA		

Table 2. Antibiotic Susceptibility Pattern of *S. aureus* Isolates[a]

Antibiotic	Sensitive	Intermediate	Resistant
Penicillin	13.1	8	78.9
Imipenem	17.8	23	59.2
Cefazolin	24.3	30	45.7
Cefalotin	41.7	39	19.3
Ceftriaxone	29	10	61
Gentamicin	13	13.4	73.6
Ciprofloxacin	7	10	83
Clindamycin	15	56	29
Azithromycin	24.5	12.5	63
Erythromycin	3.8	23	73.2
Mupirocin	60.1	21.2	18.7
Rifampicin	49.9	32	18.1
Tetracycline	19.5	34.5	46
Trimethoprim	59	13	28
Vancomycin	90.8	7.2	2
Nitrofurantoin	81	7	12

[a]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, nafcillin, and vancomycin and *S. aureus* strains showed the highest resistance to these bacteria in different reports (13-15).

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, *Luks/f-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 decolonize 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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