Biotech Health Sci. 2016 February; 3(1): e35939. doi: 10.17795/bhs-35939

Published online 2016 February 22. **Research Article**

Frequency of Fibronectin Binding Protein A and Panton-Valentine Leukocidin in Methicillin-Resistant *Staphylococcus aureus* Collected From Educational Hospitals in Qazvin, Iran

Maria Taromian,[1] Amir Peymani,[2,*] and Masoumeh Aslanimehr[2]

[1]Department of Microbiology, Zanjan Branch, Islamic Azad University, Zanjan, IR Iran
[2]Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, IR Iran

*Corresponding author: Amir Peymani, Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, IR Iran. Tel: +98-2813324971, Fax: +98-2813324971, E-mail: a.peymani@gmail.com

Received 2016 January 2; **Accepted** 2016 January 11.

Abstract

Background: *Staphylococcus aureus* is one of the most important organisms involved in nosocomial infection acquired by patients. In recent years, the appearance of methicillin-resistant *S. aureus* (MRSA) has turned the treatment of these infections into a serious challenge. Surface proteins, such as fibronectin binding proteins (FnBP), and the ability to produce Panton-Valentine leukocidin (PVL) are important factors in pathogenesis of this organism.

Objectives: The purpose of this study was to determine the prevalence of disease-associated genes in the clinical isolates of *S. aureus* encoding FNB and PVL, collected from the educational hospitals of Qazvin, Iran.

Patients and Methods: This was a descriptive, cross-sectional study in which a total of 103 isolates of methicillin-resistant *S. aureus* were collected from hospitalized patients in teaching hospitals of Qazvin, during 2013 - 2014. Initially, the identification of isolates was performed according to the standard laboratory methods, followed by confirming the presence of the *femA* gene, a gene specific to *S. aureus*. Later, the prevalence of virulence genes (*fnb* and *pvl*) was investigated by the PCR method, using specific primers. PCR products were sequenced to confirm the presence of the target genes.

Results: The results of this study showed that among 103 isolates of *S. aureus* resistant to methicillin, 88 isolates were positive for the presence of the *pvl* and *fnb* genes, with the fnb gene present in 86 (83.5%) isolates and the *pvl* gene only in 2 (1.9%) isolates.

Conclusions: The results of the present study indicate the presence of the *pvl* and *fnb* genes in the strains of *S. aureus* isolated from clinical specimens collected from the patients admitted to teaching hospitals in Qazvin. Considering the clinical significance of these organisms, and their potential in threatening public health systems, the identification, treatment, and infection control management of patients infected with these organisms is of prime necessity.

Keywords: Panton-Valentine Leukocidin, Fibronectin Binding Protein A, *Staphylococcus aureus*

1. Background

Staphylococcus aureus (*S. aureus*) is a facultative anaerobe, gram-positive coccus and part of the normal flora of the nose, skin, and gastrointestinal tract (1). This organism is one of the most common causes of nosocomial infections worldwide. Recently, the number of staphylococcal infections has been on the rise due to several reasons, including the spread of resistant strains, an increasing number of patients with immune deficiency, and the excessive use of aggressive medical devices such as catheters (2, 3). These organisms are involved in clinically important diseases including scalded skin syndrome, osteomyelitis, urinary tract infections, bacteremia, and toxic shock syndrome (4, 5). Although these bacteria are part of the normal flora of the human body in healthy people, resistance to antibacterial agents can lead to serious infections and even death. Methicillin-resistant *S. aureus* (MRSA) causes a variety of suppurative infections, which are resistant to antibiotic treatment. These organisms are unaffected by a penicillinase-resistant group of beta-lactam antibiotics (methicillin, nafcillin, and oxacillin) and cephalosporins, leading to redundancy of these antibacterial agents in treating infections caused by organisms (2). The prevalence of MRSA is high in nosocomial infections acquired at patient care centers, particularly in patients with open wounds and patients with immune deficiencies (6). The virulence of *S. aureus* is due to various factors such as surface adhesion molecules. Several studies have shown that surface proteins, such as fibronectin, play a crucial role in invasion of this organism to eukaryotic cells (7). The role and importance of attachment factors in a wide range of diseases caused by *S. aureus* has been studied, and fibronectins have been proven to play a significant role in tissue establishment of these organisms and development of serious diseases, such as keratitis and toxic arthritis, as

well as in colonization on the surface of medical devices used for patients (8, 9). These surface proteins act as adhesins and have the ability to bind to extracellular matrix proteins of epithelial and endothelial surfaces. Fibronectin is secreted in the early phase of infection and specifically binds to extracellular matrix proteins of host cells. According to several studies, these proteins are involved in adhesion to damaged heart valves and increase the intracellular entry of the organisms through epithelial cells (10-12). Another virulence factor in *S. aureus* is Panton-Valentine leukocidin, which is considered a lethal factor for human neutrophils (13). These toxins often attack macrophages and polymorphonuclears and consist of a two-component protein (33 kDa and 34 kDa) that can be separated by electrophoresis into two fractions; both inactive alone. These two components of PVL have antigenic properties and can be modified into a toxoid. This toxin resists phagocytosis, increasing the invasive potential of *S. aureus* (14, 15). PVL is the only staphylococcal toxin that exclusively affects the leukocytes. In fact, this toxin can be considered an important virulence factor of *S. aureus*, as it has the ability to destroy leukocytes and eventually reduces the number of polymorphonuclears in the host's body (16, 17). Considering the importance of *S. aureus* isolates in developing clinically important diseases, in the present study, the frequency of two genes encoding FNB and PVL as two significant virulence factors was investigated among the bacterial isolates collected from patients admitted to the teaching hospital in Qazvin. Identification of these factors provides healthcare authorities with valuable information to effectively treat infections, control, and eradicate these organisms in hospital environments.

2. Objectives

The purpose of this study was to determine the prevalence of disease-associated genes in the clinical isolates of *S. aureus* encoding the FNB and PVL virulence factors, collected from the educational hospitals of Qazvin.

3. Patients and Methods

3.1. Bacterial Isolation and Species Identification

In this cross-sectional study, a total of 103 isolates of *S. aureus* were collected from different clinical specimens of inpatients admitted to several teaching hospitals in Qazvin. These isolates were obtained from patients admitted to intensive care units (ICUs), internal medicine, neonatal and general surgery wards. Written informed consent was obtained from all subjects enrolled in this study. Initially, all isolates were identified using standard microbiological and laboratory methods, including growth on blood agar and type of hemolysis, gram stain, catalase test, slide and tube coagulase test, mannitol salt agar, and DNase test (18). Later, all isolates were identified by detection of the *femA* gene, which is specific to *S. aureus*.

3.2. Evaluation of Phenotypic Resistance to Methicillin

The disk diffusion method and agar-screening test were applied to identify MRSA. According to the clinical and laboratory standards institute (CLSI) guidelines, in the first method, the colonies of a 24 hour bacterial culture were added to normal saline to prepare a concentration equal to the turbidity present in the 0.5 McFarland standard tubes. Using the direct method, bacteria were cultured on Muller-Hinton agar medium, antibiotic disks (oxacillin 1 μg/mL; cefoxitin 30 μg/mL) were applied, and subsequently the agar plate was incubated at 37°C for 24 hours. After 24 hours following incubation, the diameter of the inhibition zones was measured and analyzed. A diameter of 22 mm for cefoxitin was considered as susceptible, while a diameter of 21 mm was considered resistant. For the oxacillin salt agar screening test, a Mueller Hinton agar medium containing 4% salt and 6 μg/mL oxacillin was made, followed by preparation of fresh bacterial culture equal to the 0.5 McFarland standard tube prepared using the direct method. Plates were placed on papers divided into 12 equal areas, followed by point cultivation of bacterial suspensions onto each area using sterile swabs. Twelve different isolates were cultured on each plate. Plates were incubated at 37°C for 24 hours. Later, the presence or absence of bacterial growth in the medium was assessed (19).

3.3. Detection of fnb and pvl Genes in MRSA Isolates

Isolation of *fnb* (encoding Fibronectin) and *pvl* (encoding Panton-Valentine leukocidin) genes was performed by total DNA extraction using a commercial extraction kit for gram-positive bacteria (Genomic DNA Extraction Kit, Bioneer Inc., South Korea), according to the manufacturer's instructions. The DNA concentrations of the extracted samples were measured with a Nano Drop™ spectrophotometer using an absorbance ratio of A260/280. Later, the PCR technique was performed using specific primers for *femA*, *fnb*, and *pvl* genes (Table 1) (20-22).

Amplification reactions were prepared in a total volume of 25 μL (24 μL of PCR master mix plus 1 μL of template DNA), including 50ng of genomic DNA, 5.0U of *Taq DNA polymerase*, 10 mM dNTP mix at a final concentration of 0.2 mM, 50 mM $MgCl_2$ at a final concentration of 1.5 mM, 10 pM of each primer, and 1X PCR buffer (final concentration). PCR amplifications were applied in a thermocycler (Applied Biosystems, USA) as follows: initial denaturation temperature (96°C for 5 minutes) and 35 thermal cycles of 1 minute at 94°C, 1 minute at specific annealing temperature for each primer (58°C for *femA* gene, 55°C for *fnb* gene, and 56°C for *pvl* gene), and 1 minute at 72°C. A final extension step of 7 minutes at 72°C was performed. PCR products were electrophoresed on

1% agarose gel at 100 volts and later stained with ethidium bromide solution, and finally visualized in a gel documentation system (UVtec, UK). The purified PCR products were sequenced by the Macrogen Company (Seoul, South Korea) and the sequence alignment and analysis were performed online, using the BLAST program of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3.4. Statistical Analysis

Data were summarized using mean ± SD (standard deviation), proportional frequency and confidence interval for microbiological, clinical, and demographic characteristics. All analyses were carried out using a statistical software package, SPSS for Windows version 16.0 (Chicago, IL, USA).

4. Results

Bacterial isolates of the present study were collected from different clinical samples including sputum (35%), wound (24.3%), blood (20.4%), urine (10.7%), and catheter (9.7%). Among these 37 samples (35.9%) were taken from women and 66 samples (64.1%) from men. Isolates were obtained from patients admitted to internal medicine (56) (54.4%), intensive care units (19) (18.4%), neonatal (21) (20.4%), and general surgery wards (7) (6.8%). All 103 isolates were positive for the presence of the *femA* gene, confirming that all isolates were *S. aureus*. The frequency of the *fnb* gene was 83.5%, a value mainly due to the organisms isolated from sputum samples (35%). Also, two isolates (1.9%), isolated from wound infections and sputum samples, were positive for the presence of *pvl* genes (Table 2).

Table 1. Primers Used in PCR to Detect *fnb*, *pvl* and *femA* Genes

Targets	Primer Sequences (5' - 3')	Reference
fnb		(20)
	F: CACAACCAGCAAATATAG	
	R: CTGTGTGGTAATCAATGTC	
pvl		(21)
	F: AGTGAACTTATCTTTCTATTGAAAAA-CACTC	
	R: GCATCAASTGTATTGGATAGCAAAAGC	
femA		(22)
	F: AAAAAAGCACATAACAAGCG	
	R: GATAAAGAAGAAACCAGCAG	

Table 2. Prevalence of *pvl* and *fnb* Genes Among 103 Isolates of *S. aureus*, Regarding Clinical Source and Hospital Wards[a]

Variables	Genes	
	fnb	*pvl*
Hospital wards		
Internal medicine	47 (45.6)	1 (1)
ICU	17 (16.5)	1 (1)
Neonatal disease	17 (16.5)	N
Surgery	5 (4.8)	N
Clinical specimens		
Sputum	31 (30.1)	1 (1)
Blood	20 (19.4)	N
Wound	17 (16.5)	1 (1)
Urine	10 (9.7)	N
Catheter	8 (7.8)	N

Abbreviations: ICU, intensive care unit; N, negative.
[a]Data are expressed as No. (%) or mean ± SD.

5. Discussion

Currently, *S. aureus* is one of the most important pathogens in humans and is considered a major bacterial agent that is involved in the development of nosocomial infections. According to many studies, a long hospital stay is considered a risk factor development of staphylococcal infections (7). Patients infected with MRSA strains stay longer in hospitals compared to those who are infected with methicillin-susceptible *S. aureus* (MSSA). A long stay in a hospital makes patients with staphylococcal infections more susceptible to the development of severe infections of internal tissues such as septicemia and osteomyelitis. In addition, the mortality rate in patients with MRSA is significantly higher compared with patients infected with methicillin-susceptible isolates (5). The presence of *fnb* gene in *S. aureus* provides the organisms with the initial attachment and entry into epithelial cells (11). Also, the presence of PVL in *S. aureus* increases the pathogenicity of this organism, leading to the establishment of severe diseases including pneumonia and soft tissue infections (13). Based on the results of the present study, among 103 methicillin-resistant *S. aureus* isolates, the frequency of *fnb* and *pvl* genes were calculated as 83.5% and 1.9%, respectively. These results showed the low prevalence rate of the PVL toxin among clinical isolates of MRSA. This result has also been reported in some studies in which MRSA strains were positive for the presence of the *pvl* gene (23-25). In a recent study from Iran, Motamedi et al. (18) reported that the *pvl* gene was positive in 6% of *S. aureus* isolates, which were all negative for the *mecA* gene. Another study in Iran by Khosravi et al. (26), which was reported, showed that of 95 isolates of *S. aureus* collected from hospitalized burn patients, the presence of the *pvl* gene was confirmed in 6 (7.2%) MRSA strains and 4 (33.3%) MSSA strains, indicating a total PVL frequency of 10.5% among the isolates tested in their study. Chi et al. (27), studied 16 isolates of *S. aureus* collected from children in Taiwan with staphylococcal toxic shock syndrome (6 isolates) and scalded skin syndrome (10 isolates) for the presence of the *fnb* gene. They reported that the *fnb* gene was present in all 16 isolates, and the presence of the *pvl* gene was only confirmed in 4 isolates (67%). In another study by Neela et al. (28), 450 MRSA isolates were studied, and a frequency of 100% was found for the *fnb* gene, whereas no *pvl* gene was detected. Also, Rasmussen et al. (29), reported that all 46 nasal MSSA isolates studied were positive for the presence of the *fnb* gene, while none were positive for the *pvl* gene. In addition, the isolates obtained from patients with invasive staphylococcal infections showed a frequency of 100% for the presence of the *fnb* gene and a low frequency (1%) for the presence of the *pvl* gene. Peacock et al (23), demonstrated that of 178 samples taken from *S.aureus* carriers, 152 samples (98%) were *fnb* positive and 6 samples (4%) carried the *pvl* gene. In conclusion, the findings of this study showed that *S. aureus* isolates collected from patients in teaching hospitals of Qazvin were considerably positive for the presence of *fnb* and *pvl* genes. The co-existence of these two virulence factors plays a significant role in the invasion, pathogenesis, and the spread of these strains. Therefore, the application of an effective strategy to control infections, especially in hospitals, and proper management of treatment is required to prevent further spread of such organisms.

Acknowledgments

The authors would like to thank the acting head, and also other staff, of the cellular and molecular research center, Qazvin University of Medical Sciences, for their assistance with completing this research project.

Footnote

Authors' Contribution: Amir Peymani: conception and design of the study; Amir Peymani and Maria Taromian: laboratory work; Masoumeh Aslanimehr: data analysis and interpretation.

References

1. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev.* 2015;**28**(3):603–61. doi: 10.1128/CMR.00134-14. [PubMed: 26016486]
2. Woods C, Colice G. Methicillin-resistant *Staphylococcus aureus* pneumonia in adults. *Expert Rev Respir Med.* 2014;**8**(5):641–51. doi: 10.1586/17476348.2014.940323. [PubMed: 25030040]
3. Bereket W, Hemalatha K, Getenet B, Wondwossen T, Solomon A, Zeynudin A, et al. Update on bacterial nosocomial infections. *Eur Rev Med Pharmacol Sci.* 2012;**16**(8):1039–44. [PubMed: 22913154]
4. Calfee DP. The epidemiology, treatment, and prevention of transmission of methicillin-resistant *Staphylococcus aureus*. *J Infus Nurs.* 2011;**34**(6):359–64. doi: 10.1097/NAN.0b013e31823061d6. [PubMed: 22101629]
5. Otter JA, French GL. Community-associated meticillin-resistant Staphylococcus aureus strains as a cause of healthcare-associated infection. *J Hosp Infect.* 2011;**79**(3):189–93. doi: 10.1016/j.jhin.2011.04.028. [PubMed: 21741111]
6. Chen CJ, Huang YC. New epidemiology of Staphylococcus aureus infection in Asia. *Clin Microbiol Infect.* 2014;**20**(7):605–23. doi: 10.1111/1469-0691.12705. [PubMed: 24888414]
7. Tong SY, Chen LF, Fowler VG, Jr. Colonization, pathogenicity, host susceptibility, and therapeutics for *Staphylococcus aureus*: what is the clinical relevance? *Semin Immunopathol.* 2012;**34**(2):185–200. doi: 10.1007/s00281-011-0300-x. [PubMed: 22160374]
8. Wang M, Zhang Y, Li B, Zhu J. Construction of scFv that bind both fibronectin-binding protein A and clumping factor A of *Stapylococcus aureus*. *Res Vet Sci.* 2015;**100**:109–14. doi: 10.1016/j.rvsc.2015.02.012. [PubMed: 25910693]
9. Rasigade JP, Moulay A, Lhoste Y, Tristan A, Bes M, Vandenesch F, et al. Impact of sub-inhibitory antibiotics on fibronectin-mediated host cell adhesion and invasion by *Staphylococcus aureus*. *BMC Microbiol.* 2011;**11**:263. doi: 10.1186/1471-2180-11-263. [PubMed: 22168812]
10. Edwards AM, Potts JR, Josefsson E, Massey RC. Staphylococcus aureus host cell invasion and virulence in sepsis is facilitated by the multiple repeats within FnBPA. *PLoS Pathog.* 2010;**6**(6):e1000964. doi: 10.1371/journal.ppat.1000964. [PubMed: 20585570]
11. Piroth L, Que YA, Widmer E, Panchaud A, Piu S, Entenza JM, et al. The fibrinogen- and fibronectin-binding domains of *Staphylococcus aureus* fibronectin-binding protein A synergistically promote endothelial invasion and experimental endocarditis. *Infect Immun.* 2008;**76**(8):3824–31. doi: 10.1128/IAI.00405-08. [PubMed: 18541660]

12. Heying R, van de Gevel J, Que Y-A, Moreillon P, Beekhuizen H. Fibronectin-binding proteins and clumping factor A in *Staphylococcus aureus* experimental endocarditis: FnBPA is sufficient to activate human endothelial cells. *Thromb Haemost.* 2007;**97**(4):617–26. [PubMed: 17393025]
13. Shrestha B. Review on Panton Valentine leukocidin toxin carriage among *Staphylococcus aureus*. *J Nepal Health Res Counc.* 2013;**11**(25):305–12. [PubMed: 24908537]
14. Ritz N, Curtis N. The role of Panton-Valentine leukocidin in Staphylococcus aureus musculoskeletal infections in children. *Pediatr Infect Dis J.* 2012;**31**(5):514–8. doi: 10.1097/INF.0b013e31824f18cb. [PubMed: 22327874]
15. Lo WT, Wang CC. Panton-Valentine leukocidin in the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. *Pediatr Neonatol.* 2011;**52**(2):59–65. doi: 10.1016/j.pedneo.2011.02.008. [PubMed: 21524624]
16. Shrestha B, Singh W, Raj VS, Pokhrel BM, Mohapatra TM. High prevalence of Panton-Valentine leukocidin (PVL) genes in nosocomial-acquired *Staphylococcus aureus* isolated from tertiary care hospitals in Nepal. *Biomed Res Int.* 2014;**2014**:790350. doi: 10.1155/2014/790350. [PubMed: 25045702]
17. Beyrouthy R, Hamze M, Hleis S, Mallat H, Dabboussi F. Panton-Valentine leukocidin producing *Staphylococcus aureus* nasal carriage, in North-Lebanon. *Med Mal Infect.* 2013;**43**(9):386–90. doi: 10.1016/j.medmal.2013.07.005. [PubMed: 23973399]
18. Motamedi H, Rahmat Abadi SS, Moosavian SM, Torabi M. The Association of Panton-Valentine leukocidin and mecA Genes in Methicillin-Resistant *Staphylococcus aureus* Isolates From Patients Referred to Educational Hospitals in Ahvaz, Iran. *Jundishapur J Microbiol.* 2015;**8**(8):e22021. doi: 10.5812/jjm.22021v2. [PubMed: 26468365]
19. Wayne P. *Performance standards for antimicrobial susceptibility testing.* USA: Clinical and Laboratory Standards Institute (CLSI); 2013. pp. M100–S23.
20. Delgado S, Garcia P, Fernandez L, Jimenez E, Rodriguez-Banos M, del Campo R, et al. Characterization of *Staphylococcus aureus* strains involved in human and bovine mastitis. *FEMS Immunol Med Microbiol.* 2011;**62**(2):225–35. doi: 10.1111/j.1574-695X.2011.00806.x. [PubMed: 21477005]
21. Netsvyetayeva I, Fraczek M, Piskorska K, Golas M, Sikora M, Mlynarczyk A, et al. *Staphylococcus aureus* nasal carriage in Ukraine: antibacterial resistance and virulence factor encoding genes. *BMC Infect Dis.* 2014;**14**:128. doi: 10.1186/1471-2334-14-128. [PubMed: 24597648]
22. Arakere G, Nadig S, Swedberg G, Macaden R, Amarnath SK, Raghunath D. Genotyping of methicillin-resistant *Staphylococcus aureus* strains from two hospitals in Bangalore, South India. *J Clin Microbiol.* 2005;**43**(7):3198–202. doi: 10.1128/JCM.43.7.3198-3202.2005. [PubMed: 16000435]
23. Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, et al. Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. *Infect Immun.* 2002;**70**(9):4987–96. [PubMed: 12183545]
24. San Juan JA, Cabugao DBG, Dabbay BRF, Cabrera EC. Occurrence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among the Health Workers of Rizal Provincial Hospital and Characterization for the Presence of luks-lukf PVL Gene. *Philippine Journal of Science.* 2012;**141**(2):157–63.
25. Rebollo-Pérez J, Ordoñez-Tapia C, Herazo-Herazo C, Reyes-Ramos N. Nasal carriage of Panton Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* in healthy preschool children. *Revista de Salud Pública.* 2011;**13**(5):824–32. [PubMed: 22634949]
26. Khosravi AD, Hoveizavi H, Farshadzadeh Z. The prevalence of genes encoding leukocidins in *Staphylococcus aureus* strains resistant and sensitive to methicillin isolated from burn patients in Taleghani Hospital, Ahvaz, Iran. *Burns.* 2012;**38**(2):247–51. doi: 10.1016/j.burns.2011.08.002. [PubMed: 21924558]
27. Chi CY, Wang SM, Lin HC, Liu CC. A clinical and microbiological comparison of *Staphylococcus aureus* toxic shock and scalded skin syndromes in children. *Clin Infect Dis.* 2006;**42**(2):181–5. doi: 10.1086/498901. [PubMed: 16355327]
28. Neela V, Mohd Zafrul A, Mariana NS, van Belkum A, Liew YK, Rad EG. Prevalence of ST9 methicillin-resistant *Staphylococcus aureus* among pigs and pig handlers in Malaysia. *J Clin Microbiol.* 2009;**47**(12):4138–40. doi: 10.1128/JCM.01363-09. [PubMed: 19812280]
29. Rasmussen G, Monecke S, Ehricht R, Soderquist B. Prevalence of clonal complexes and virulence genes among commensal and invasive *Staphylococcus aureus* isolates in Sweden. *PLoS One.* 2013;**8**(10):e77477. doi: 10.1371/journal.pone.0077477. [PubMed: 24130888]