Journal of Infection and Public Health (2015) 8, 364—368

http://www.elsevier.com/locate/jiph

Prevalence of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* infections in a Saudi Arabian hospital

Ali M. Bazzi[a], **Ali A. Rabaan**[b], **Mahmoud M. Fawarah**[a], **Jaffar A. Al-Tawfiq**[c,d,*]

[a] *Microbiology Laboratory, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia*
[b] *Molecular Diagnnostic Laboratory, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia*
[c] *Specialty Internal Medicine, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia*
[d] *Indiana University School of Medicine, IN, USA*

Received 2 November 2014; received in revised form 10 January 2015; accepted 23 January 2015

KEYWORDS
Panton-Valentine leukocidin;
Methicillin-susceptible *Staphylococcus aureus*;
MSSA

Summary Panton-Valentine leukocidin (PVL) is a two-component toxin associated with the toxicity and virulence of *Staphylococcus aureus*. The presence of PVL is well documented in community-acquired methicillin-resistant *S. aureus* (CA-MRSA) and is observed in methicillin-susceptible *S. aureus* (MSSA) with variable prevalence. We assessed the prevalence of PVL in a sample of 93 MSSA patients in a healthcare facility in Eastern Saudi Arabia using real-time PCR for lukSF-PV genes. The presence or absence of PVL was correlated with age, gender, hospitalization status, infection site and antibiotic resistance. PVL was detected in 28 (30%) patient samples. PVL was associated with a greater likelihood of resistance to trimethoprim—sulfamethoxazole (a resistance of 39.2% of PVL-positive isolates compared to 6.1% of PVL-negative isolates) ($p < 0.0007$). These results suggest a significant prevalence of PVL expression in MSSA strains in the study population and call for monitoring of and surveillance programs for PVL status and the selection of appropriate antibiotic treatments.

* Corresponding author at: P.O. Box 76, Room A-428-2, Building 61, Dhahran Health Center, Saudi Aramco, Dhahran 31311, Saudi Arabia. Tel.: +966 13 877 9748; fax: +966 13 877 3790.
E-mail addresses: jaffar.tawfiq@jhah.com, jaltawfi@yahoo.com (J.A. Al-Tawfiq).

http://dx.doi.org/10.1016/j.jiph.2015.01.010

Introduction

Staphylococcus aureus are nasal, commensal Gram-positive cocci, which colonize in 20—30% of the human population [1], as well as livestock and domestic animals [2,3]. As a human pathogen, *S. aureus* causes infections ranging from mild skin and soft tissue infections to life-threatening sepsis, pneumonia, and toxic shock syndrome. *S. aureus* pathophysiology depends on the presence of virulence factors, including those present on the cell surface and secreted factors. One virulence factor associated with *S. aureus* toxicity is the Panton-Valentine leukocidin (PVL), a two-component toxin that acts by forming pores in the mitochondria [4]. The dual leukocidin PVL toxin components, LukS and LukF, are encoded by the adjacent prophage *lukS* and *lukF* genes [5,6]. PVL reduces immune resistance in a number of ways; for example, it causes neutrophil lysis or apoptosis [4,7] and targets complement receptors [8]. In humans, PVL is associated with skin and soft tissue infections (SSTI), bone and joint infections and necrotizing pneumonia [9]. PVL has been linked to exacerbation of bone loss in osteomyelitis [10] and is proposed as an important virulence factor in keratitis associated with *S. aureus* infection [11].

The prevalence of PVL in methicillin-resistant *S. aureus* (MRSA) is well documented. It is highly expressed in community-acquired (CA)-MRSA strains. However, PVL is expressed in healthcare-associated (HA)-MRSA. PVL is a relatively stable marker of CA-MRSA and is associated in particular with the staphylococcal cassette chromosomes (SCCmec) types IV and V [5,6,12]. The role of PVL in CA-MRSA virulence is debated. In humans, it is associated with increased virulence [6], but animal studies have yielded conflicting results, with a possible immunomodulatory role suggested beyond the cytotoxic effects [13—15].

PVL has also been observed in methicillin-susceptible *S. aureus* (MSSA) strains. Although the epidemiology has not been as extensively established as in MRSA, recently, interest has increased in this field of research. PVL prevalence in MSSA infections varies between countries, from low levels (0.7—2.9%) in Northern Spain, Ireland and Portugal [16—18] to 37% in New Zealand and even higher in African countries, such as Nigeria [19], Cameroon, Madagascar, Morocco, Niger, and Senegal [20]. PVL-positive MSSA has been associated with SSTI [21,22] and cases of necrotizing pneumonia [23,24]. Risk factors for PVL-positive MSSA include Pacific ethnicity, young age, SSTI diagnosis, community-acquired onset of infection, need for surgical intervention, prior hospitalization and smoking [21,25,26].

Because of the wide variation in incidence of PVL-positive MSSA between countries, it is important to characterize MSSA PVL prevalence and risk factors in Saudi Arabia. No such comprehensive analysis has been performed previously, although an isolated case of PVL-positive MSSA was reported in a child with acute osteomyelitis [27] and among 37.6% of 101 MRSA isolates in Jeddah [28]. This study presents data on PVL prevalence in 93 patients with MSSA from a healthcare facility in the Eastern Province of Saudi Arabia.

Materials and methods

Bacterial isolates

A total of 93 MSSA clinical isolates were randomly collected from the Dhahran Health Center, Microbiology Section, from January until December 2013. These specimens were obtained at the request of the attending physician for clinical reasons. The identification of the strains and antibiotics profiles were performed using the VITEK II system. The isolates were sent to the molecular diagnostic laboratory for PVL gene testing. Each isolate was isolated from a different patient.

DNA extraction

Genomic DNA was extracted using the Roche MagNa pure compact nucleic acid isolation kit I, DNA bacteria protocol, according to the manufacturer instructions. Briefly, each strain was resuspended in 0.2 ml of 0.85% saline.

Detection of lukS PV gene

The extracted DNA was screened with the TIB-MOLBIOL LightMix® CA-MRSA PCR kit, Cat# 40-0325-16.

Clinical data

Electronic clinical records were reviewed to ascertain the following demographic data: patient age, gender, and hospitalization.

Results

MSSA isolates were obtained from a total of 93 patients with an age range of 11 months to 99 years. Most infections (72%) were obtained from sputum, blood, semen or urine samples, catheter-related infections, wounds or abscesses. The patients were

Table 1 Antibiotic-resistance pattern of all isolates and PVL positive and PVL negative isolates.

	Number resistance (%), $N = 93$	Resistance (%) among PVL+, $N = 28$	Number resistance (%) among PVL−, $N = 65$
Penicillin	82 (88.17)	28 (100)	54 (83)
Oxacillin	0 (0)	0 (0)	0 (0)
Gentamicin	1 (1.07)	1 (3.6)	0 (0)
Ciprofloxacin	16 (17.02)	7 (25)	9 (13.8)
Levofloxacin	15 (16.3)	7 (25)	8 (12.3)
Moxifloxacin	15 (16.3)	7 (25)	8 (12.3)
Erythromycin	9 (9.6)	3 (10.7)	6 (9.2)
Clindamycin	2 (2.15)	0 (0)	2 (3)
Quinupristin	0 (0)	0 (0)	0 (0)
Linezolid	0 (0)	0 (0)	0 (0)
Vancomycin	0 (0)	0 (0)	0 (0)
Tetracycline	7 (8.6)	2 (7.1)	5 (7.6)
Tigecycline	0 (0)	0 (0)	0 (0)
Nitrofurantoin	0 (0)	0 (0)	0 (0)
Rifampicin	0 (0)	0 (0)	0 (0)
Trimethoprim—sulfamethoxazole	14 (15)	12 (42.8)	2 (3)

divided almost equally between those who were (48.4%) or were not (51.6%) hospitalized during the previous year. The antibiogram of the isolates is shown in Table 1. Most isolates were resistant to penicillin (88.75%), and all were susceptible to oxacillin, quinupristin, linezolid, tigecycline, nitrofurantoin and rifampicin.

PVL presence or absence was confirmed by real-time PCR for *lukSF-PV* genes. PVL was detected in 28 of 93 samples (30%). Table 2 shows the results of the univariate analysis to compare features of PVL-positive to PVL-negative MSSA. A significantly higher proportion of PVL-negative subjects were aged between 50 and 59 years (33.9%) compared to the PVL-positive patients (10.7%) ($p = 0.023$) (Table 2). Of all PVL positive patients, seven (25%) were aged 0—9 years, two (7.1%) were aged 10—19 years, six (21.4%) were aged 20—29 years, four (14.2%) were aged 40—49 years, and three (10.7%) were aged 50—59 years (Table 2).

Resistance to trimethoprim—sulfamethoxazole showed a strong association with PVL-positivity, and 39.2% of PVL-positive isolates were resistant compared to 6.1% of PVL-negative isolates ($p = 0.0007$) (Table 2). No other antibiotic resistance profile showed any association with PVL positivity (data not shown). Gender, hospitalization status or site of infection was not associated with PVL positivity (Table 2).

Discussion

The results showed that the prevalence of PVL in MSSA infections in this study was 30%. This finding is similar to reports for PVL-positive MSSA prevalence in Auckland, New Zealand [21] and in the Arkhangelsk region of Russia [29]. It is significantly higher than the reported prevalence in Northern Spain, Queensland, Australia, Ireland and Portugal [16—18,30], and lower than the rate in African countries, in particular Cameroon, Niger, and Senegal [20].

Previous studies showed that risk factors for PVL presence in MSSA strains include younger age and prior hospitalization [21,25,30]. Similarly, in the current study, PVL positivity is associated with a younger age (0—29 years) and prior hospitalization.

For antibiotic resistance, the majority (88.75%) of isolates were resistant to penicillin. Of the 15 isolates that were resistant to trimethoprim—sulfamethoxazole, 11 were PVL-positive. This represents 39.2% of PVL-positive isolates compared to 6.1% of PVL-negative isolates. Trimethoprim-sulfamethoxazole resistance may represent the spread of a specific clone in our locality. This trend for the association of trimethoprim—sulfamethoxazole with PVL should be further examined.

The current study has a relatively small sample size and did not characterize samples according to individual infection groups, nor did it consider factors such as ethnicity or social deprivation, which influence PVL association with MSSA according to other studies [21]. Therefore, the sample size does not produce (with confidence) a significant association with the investigated risk factors. Further studies should increase the sample size and consider other potential contributory factors and should investigate the association between

Table 2 Univariate analysis of patients with PVL-positive MSSA infection versus PVL-negative MSSA infection.

Characteristic	Number (%) of all patients ($N = 91$)	No. (%) of PVL-positive patients ($N = 28$)	No. (%) of PVL-negative patients ($N = 65$)	OR (95% CI)	p value
Female gender	40 (42)	13 (46.0)	27 (41.5)	0.83 (0.31—2.15)	0.806
Age (year)					
0—9	14 (15)	7 (25)	7 (10.7)	2.76 (0.79—9.73)	0.168
10—19	8 (8.6)	2 (7.1)	6 (9.2)	0.93 (0.17—5.15)	1.000
20—29	11 (11.8)	6 (21.4)	5 (7.6)	3.42 (0.83—14.10)	0.119
30—39	5 (5.3)	2 (7.1)	2 (3.6)	2.46 (0.32—18.54)	0.579
40—49	13 (13.9)	4 (14.2)	8 (12.3)	1.40 (0.37—5.32)	0.726
50—59	24 (25.4)	3 (10.7)	22 (33.9)	0.18 (0.038—0.83)	0.025
60—69	8 (8.6)	1 (3.6)	7 (10.7)	0.36 (0.041—3.19)	0.668
70—79	6 (6.4)	1 (3.6)	5 (7.6)	0.44 (0.060—5.34)	1.0000
80—89	0 (0)	0 (0)	0 (0)	NA	NA
90—99	4 (5)	2 (7.1)	3 (4.6)	0.77 (0.076—7.78)	1.000
Hospitalization	43 (43.3)	13 (46.4)	32 (49.2)	0.89 (0.32—2.20)	0.809
Invasive infection	67 (67.7)	22 (78.5)	45 (69.2)	1.63 (0.51—5.05)	0.582
Trimethoprim—sulfamethoxazole resistant	15 (15)	11 (39.2)	4 (6.1)	0.094 (0.023—0.39)	0.0007

NA: not applicable.

trimethoprim-sulfamethoxazole resistance and PVL. In addition, it is necessary to assess large numbers of patients, with an emphasis on risk factors/groups. These factors include close contact, such as sports and crowding, and skin abrasion, cleanliness, or item sharing. The authors advocate standardized surveillance procedures for *S. aureus* infections in the region and mindfulness of the potential contribution of PVL to MSSA as well as MRSA virulence. Trends indicated by this study suggest that the presence of PVL should influence the selection of antibiotic treatment, particularly in the case of trimethoprim—sulfamethoxazole.

Financial support

None.

Conflict of interest

None of the authors has a conflict of interest to declare.

Acknowledgments

The authors wish to acknowledge the use of Johns Hopkins Aramco Helathcare (JHAH) facilities for the data and study for this paper. The opinions expressed in this article are those of the authors and not necessarily of JHAH.

References

[1] vanBelkum A, Melles DC, Nouwen J, van Leeuwen WB, van Wamel W, Vos MC, et al. Co-evolutionary aspects of human colonisation and infection by *Staphylococcus aureus*. Infect Genet Evol Infect Genet Evol 2009;9(January (1)):32—47.

[2] Lindsay JA. Staphylococcus aureus genomics and the impact of horizontal gene transfer. Int J Med Microbiol 2014;304(March (2)):103—9.

[3] Peton V, Le Loir Y. *Staphylococcus aureus* in veterinary medicine. Infect Genet Evol 2014;January (21):602—15.

[4] Genestier AL, Michallet MC, Prévost G, Bellot G, Chalabreysse L, Peyrol S, et al. *Staphylococcus aureus* Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. J Clin Invest 2005;115(November (11)):3117—27.

[5] Chambers HF. Staphylococcal purpura fulminans: a toxin-mediated disease? Clin Infect Dis 2005;40(April (7)):948—50.

[6] Otto M. Community-associated MRSA: what makes them special? Int J Med Microbiol 2013;303(August (6—7)): 324—30.

[7] Löffler B, Hussain M, Grundmeier M, Brück M, Holzinger D, Varga G, et al. *Staphylococcus aureus* Panton-Valentine

leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog 2010;6(1):1.
[8] Spaan AN, Henry T, van Rooijen WJ, Perret M, Badiou C, Aerts PC, et al. The staphylococcal toxin Panton-Valentine leukocidin targets human C5a receptors. Cell Host Microbe 2013;13(May (5)):584–94.
[9] Shallcross LJ, Fragaszy E, Johnson AM, Hayward AC. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. Lancet Infect Dis 2013;13(January (1)):43–54, http://dx.doi.org/10.1016/S1473-3099(12)70238-4.
[10] Jin T, Zhu YL, Li J, Shi J, He XQ, Ding J, et al. Staphylococcal protein A, Panton-Valentine leukocidin and coagulase aggravate the bone loss and bone destruction in osteomyelitis. Cell Physiol Biochem 2013;32(2):322–33.
[11] Sueke H, Shankar J, Neal T, Winstanley C, Tuft S, Coates R, et al. lukSF-PV in *Staphylococcus aureus* keratitis isolates and association with clinical outcome. Invest Ophthalmol Vis Sci 2013;54(May (5)):3410–6, http://dx.doi.org/10.1167/iovs.12-11276.
[12] Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 2003;9(August (8)):978–84.
[13] Day SR, Moore CM, Kundzins JR, Sifri CD. Community-associated and healthcare-associated methicillin-resistant *Staphylococcus aureus* virulence toward *Caenorhabditis elegans* compared. Virulence 2012;3(November (7)):576–82.
[14] Wu K, Zhang K, McClure J, Zhang J, Schrenzel J, Francois P, et al. A correlative analysis of epidemiologic and molecular characteristics of methicillin-resistant *Staphylococcus aureus* clones from diverse geographic locations with virulence measured by a *Caenorhabditis elegans* host model. Eur J Clin Microbiol Infect Dis 2013;32(January (1)):33–42.
[15] Yoong P, Pier GB. Immune-activating properties of Panton-Valentine leukocidin improve the outcome in a model of methicillin-resistant *Staphylococcus aureus* pneumonia. Infect Immun 2012;80(August (8)):2894–904, http://dx.doi.org/10.1128/IAI.06360-11.
[16] Marimón JM, Villar M, García-Arenzana JM, Caba Ide L, Pérez-Trallero E. Molecular characterization of *Staphylococcus aureus* carrying the Panton-Valentine leucocidin genes in northern Spain. J Infect 2012;64(January (1)):47–53.
[17] Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S, Coleman DC. Panton-Valentine leukocidin-positive *Staphylococcus aureus* in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant *S. aureus* clones, and increasing multiresistance. J Clin Microbiol 2014;52(March (3)):859–70.
[18] Tavares A, Miragaia M, Rolo J, Coelho C, de Lencastre H, CA-MRSA/MSSA Working Group. High prevalence of hospital-associated methicillin-resistant *Staphylococcus aureus* in the community in Portugal: evidence for the blurring of community-hospital boundaries. Eur J Clin Microbiol Infect Dis 2013;32(October (10)):1269–83.
[19] Shittu A, Oyedara O, Abegunrin F, Okon K, Raji A, Taiwo S, et al. Characterization of methicillin-susceptible and -resistant staphylococci in the clinical setting: a multicentre study in Nigeria. BMC Infect Dis 2012;November (12):286.
[20] Breurec S, Fall C, Pouillot R, Boisier P, Brisse S, Diene-Sarr F, et al. Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton-Valentine leukocidin genes. Clin Microbiol Infect 2011;17(April (4)):633–9.
[21] Muttaiyah S, Coombs G, Pandey S, Reed P, Ritchie S, Lennon D, et al. Incidence, risk factors, and outcomes of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* infections in Auckland, New Zealand. J Clin Microbiol 2010;48(October (10)):3470–4.
[22] Zhao C, Liu Y, Zhao M, Liu Y, Yu Y, Chen H, et al. Characterization of community acquired *Staphylococcus aureus* associated with skin and soft tissue infection in Beijing: high prevalence of PVL+ ST398. PLoS ONE 2012;7(6):e38577.
[23] Eshwara VK, Munim F, Tellapragada C, Kamath A, Varma M, Lewis LE, et al. *Staphylococcus aureus* bacteremia in an Indian tertiary care hospital: observational study on clinical epidemiology, resistance characteristics, and carriage of the Panton-Valentine leukocidin gene. Int J Infect Dis 2013;17(November (11)):e1051–5.
[24] Thomas B, Pugalenthi A, Chilvers M. Pleuropulmonary complications of PVL-positive *Staphylococcus aureus* infection in children. Acta Paediatr 2009;98(August (8)):1372–5.
[25] Santosaningsih D, Santoso S, Budayanti NS, Kuntaman K, Lestari ES, Farida H, et al. Epidemiology of *Staphylococcus aureus* harboring the mecA or Panton-Valentine leukocidin genes in hospitals in Java and Bali, Indonesia. Am J Trop Med Hyg 2014;90(April (4)):728–34.
[26] Wardyn SE, Forshey BM, Smith TC. High prevalence of Panton-Valentine leukocidin among methicillin-sensitive *Staphylococcus aureus* colonization isolates in rural Iowa. Microb Drug Resist 2012;18(August (4)):427–33.
[27] Bukhari EE, Al-Otaibi FE, El-Hazmi MM, Somily AM. Panton-Valentine leukocidin *Staphylococcus aureus* osteomyelitis of the femur in a Saudi child. Saudi Med J 2012;33(February (2)):201–4.
[28] Moussa I, Kabli SA, Hemeg HA, Al-Garni SM, Shibl AM. A novel multiplex PCR for molecular characterization of methicillin resistant *Staphylococcus aureus* recovered from Jeddah, Kingdom of Saudi Arabia. Indian J Med Microbiol 2012;30(July–September (3)):296–301.
[29] Vorobieva V, Bazhukova T, Hanssen AM, Caugant DA, Semenova N, Haldorsen BC, et al. Clinical isolates of *Staphylococcus aureus* from the Arkhangelsk region, Russia: antimicrobial susceptibility, molecular epidemiology, and distribution of Panton-Valentine leukocidin genes. APMIS 2008;116(October (10)):877–87.
[30] Munckhof WJ, Nimmo GR, Carney J, Schooneveldt JM, Huygens F, Inman-Bamber J, et al. Methicillin-susceptible, non-multiresistant methicillin-resistant and multiresistant methicillin-resistant *Staphylococcus aureus* infections: a clinical, epidemiological and microbiological comparative study. Eur J Clin Microbiol Infect Dis 2008;27(May (5)):355–64.