

Review on Panton Valentine Leukocidin Toxin Carriage among *Staphylococcus aureus*

Shrestha B¹

¹Department of Microbiology, Tri-Chandra Campus, Tribhuvan University, Nepal.

ABSTRACT

Panton Valentine leukocidin is a toxin making pores in the polymorphonuclear cells which is a virulence factor of some strains of *Staphylococcus aureus*. Initially it was produced by methicillin susceptible *Staphylococcus aureus* only. Later with the acquisition of *mecA* gene has led it to be PVL positive methicillin resistant *Staphylococcus aureus*. Since MRSA are resistant to many antibiotics and further they produce a toxin the infections by PVL positive MRSA has become a challenge. PVL positive MRSA a virulent strain of drug resistant superbug MRSA that has spread around the world, has claimed many lives in UK, Europe, USA and Australia. Some strains of superbug attack the healthy young people and kill within 24 hrs.

PVL positive *Staphylococcus aureus* has been reported to be associated with skin and soft tissue infections however they also cause invasive infections and necrotizing pneumonia. These microorganisms known to be community associated have spread to hospitals. Hospital acquired infection by such microorganisms lead to an increase in mortality hence should be controlled before they become prevalent in hospitals.

Keywords: community acquired; hospital acquired; MRSA; MSSA; PVL.

INTRODUCTION

Panton-Valentine leukocidin (PVL) is a leukocidin toxin discovered by van de Velde in 1894. He described it as a substance that is secreted by virulent clones which lyses leukocytes. In 1932, Panton and Valentine described its link with severe abscess, its association with styes, carbuncles, and pyemic infections.¹ In 1999 Lina et al. described the association between *Staphylococcus aureus* carrying PVL genes and community acquired necrotizing pneumonia; and developed a PCR assay to detect the PVL genes.¹ Infections by PVL positive *S. aureus* have predilection to attack the skin and can be treated with antibiotics. However, in some cases PVL infections spread to the lungs causing necrotizing pneumonia, a disease that rapidly destroys lung tissue and is lethal in 75 % of cases some dying within 48 hrs.² Community acquired pneumonia by PVL carrying *S. aureus* attack the immunocompetent children and young adults with previously healthy lungs.¹

USA 300, THE HIGHLY VIRULENT STRAIN

USA 300 strain is a very virulent PVL positive methicillin susceptible *S. aureus* (MSSA) strain that can claim life in 24 hrs after the infection. USA clone is spreading in USA hospitals hence they are epidemic in some communities in at least 16 states in the USA. These highly transmissible and virulent strains carry a novel mobile genetic element, arginine catabolic mobile element encoding arginine deaminase pathway and an oligopeptide permease system that act as an additional virulence factor. They also carry genes encoding staphylococcal enterotoxin.³

PVL positive methicillin resistant *S. aureus* (MRSA) strains pose a serious risk to health due to its multiple antibiotic resistance. Treatment of infection by organisms that can pump out a potentially deadly toxin and have multiple resistance is difficult. The bug producing PVL had surfaced in 1950 but was not resistant to antibiotics, now it has developed resistance and we are at an early

Correspondence: Dr. Bidya Shrestha, Department of Microbiology, Tri-Chandra College, Tribhuvan University, Nepal. Email: b_shrestha_07@hotmail.com, Phone: 9841350850.

stage of epidemic, and it is spreading very fast. PVL strains if become widespread in hospitals, they could inflict a much greater death toll than the existing MRSA superbugs.² PVL positive MRSA has been a threat because it has been spreading outside hospitals. However, not all bacteria that produce PVL are so dangerous or difficult to treat.

PATHOGENESIS

PVL produced by *S. aureus* is a pore forming toxin which destroys white blood cells especially polymorphonuclear cell (PMN), an important defence against staphylococcal infection hence, it is a virulence factor.⁴ PVL is composed of two separate proteins LukS-PV and LukF-PV and they act synergistically to produce the toxic effect. These proteins are encoded by two genes *lukS-PV* and *lukF-PV* which are contiguous and are cotranscribed.⁵ The multi component protein toxin composed of four LukF and four LukS subunits form an octameric pore in the affected membrane. They disrupt leukocyte membranes electively, thus leading to increased virulence. Hence, PVL-carrying *S.aureus* strains are able to causes recurrent, chronic or particularly severe skin and soft-tissue infections as well as rapidly fatal pneumonia.⁵ High concentration of PVL cause the lysis of PMN and low concentration leads to apoptosis (programmed cell death) of PMN.⁶

When injected intradermally in rabbits, the purified PVL induces severe inflammatory lesions leading to capillary dilation, chemotaxis, PMN infiltration, PMN karyorrhexis, and skin necrosis.⁷

In an experiment carried out in University of Texas in Houston and Lyon University in France, scientists found that the mice that inhaled the PVL-producing *Staphylococcus* quickly developed necrotizing pneumonia, with some dying within 48 hours. PVL producing bacteria also produce higher levels of proteins that caused massive inflammation and made the bacteria more "sticky", helping them to cling to people's skin and making it easier to spread.² The protein overproduction causes the damage and helps the infection to spread. Carriage of *PVL* gene alone may not be the major virulence factor; rather factors that upregulate toxin synthesis in vivo could contribute to more severe disease and worse outcome.⁸

It has been reported that in comparison to PVL negative *S. aureus*, PVL positive *S. aureus* exhibit increased affinity for damaged airway epithelium and specifically for exposed basement membrane. They have comparatively stronger affinity for type I and IV collagens and laminin. Epithelial damage possibly due to viral infection and/or due to a PVL negative *S. aureus* product action, permit

the binding of PVL positive *S. aureus* to the exposed type I and IV collagens and laminin. The PVL toxin then produced cause the necrotizing pneumonia.⁹

EPIDEMIOLOGY

Not all people with PVL positive *S. aureus* suffer from infections because asymptomatic carrier status of PVL positive *S. aureus* also exists.¹⁰ PVL positive *S. aureus* infections are highly transmissible and can spread more readily in settings where individuals are in close physical contact or share personal items, for example, towels. Families/household, educational settings, military barracks, care homes, prison sets, gym are also considered as risk factors as overcrowding is also a risk factor.¹¹ The infections are usually associated with the presence of other risk factors like skin abrasions resulting from close contact sports (wrestling, rugby, judo), or by using contaminated articles (sharing towels, razors), poor hand hygiene, illicit drug use and damaged skin from conditions like eczema.¹⁰ In the US, outbreak of severe skin infections has been reported in school children, prison inmates, homosexual men.¹²

PVL has been estimated to be produced by less than 2% of *S. aureus* both MSSA and MRSA.¹³ Though only 2% of *S. aureus* express the toxin, nearly 90% of the strains isolated from severe skin infections (dermonecrotic lesions) express this toxin.⁴ Therefore, it is considered as an important factor in necrotizing skin infections. At least 14 strains of PVL positive *S. aureus* are known.¹⁴ PVL positive *S. aureus* are normally associated with mild skin/tissue infection, (necrotising pyogenic cutaneous infections) and occasionally with cellulitis or tissue necrosis.¹⁵ Further, these organisms have been associated with skin abscess, furunculosis.¹ PVL genes were detected in 93 % of *S. aureus* isolates from furunculosis and in 55 % of isolates from cellulites.¹ However, they also cause other severe invasive infections such as septic arthritis, bacteremia, purpura fulminans¹⁶ or severe life threatening pneumonia the community-acquired necrotising pneumonia.^{1,17,18} It has been reported that of 27 *S. aureus* isolates from community acquired pneumonia PVL gene was detected in 23 isolates.¹ The PVL positive *S. aureus* are of no particular significance in bacteremia as only 1.6 % of *S. aureus* (MSSA) from bacteremic cases has been reported to be PVL positive.¹⁹

Table 1. Prevalence of PVL positive *S. aureus* in various infections.

Infection	percentage
Furunculosis	93% ¹
Cellulitis	55% ¹
Pneumonia	85.2% ²⁷
Bacterimia	1.6% ¹⁹

SURGICAL SITE INFECTION

Surgical site infections by PVL positive *S. aureus* are often recurrent and can be severe. Hence, impact of such infections can be substantial due to the need for prolonged or repeated courses of treatment. PVL positive *S. aureus* most commonly causes pyogenic skin infections like boils and abscesses which require incision and drainage. Health Protection Agency's *Staphylococcus* Reference Unit (SRU) has reported that 65% of *S. aureus* infections associated with surgical site infection are PVL positive and one third of which were associated with recurrent episodes of infection.²⁰

CLINICAL FEATURES OF INFECTIONS

Surgical site infections are often recurrent and these include boils (furunculosis), carbuncles, folliculitis, cellulitis, purulent eyelid infection, cutaneous lesions of at least 5cm or more in diameter, pain and erythema out of proportion to severity of cutaneous findings and necrosis.¹¹

The invasive infections include necrotising pneumonia (begins with flu like illness), necrotising fasciitis, osteomyelitis, septic arthritis and pyomyositis, and purpura fulminans.¹¹

PVL positive *S. aureus* in relation to the site of infection

Different researchers from different geographical locations have reported various ranges of prevalence of PVL positive *S. aureus* in various sites of infection. In Australia PVL positive *S. aureus* has been reported to cause 90% of skin and soft tissue infection and 95% of skin and soft tissue infections were presented as furunculosis.²¹ In the UK, 1.6% of *S. aureus* has been reported positive for the PVL.¹³ On more isolates selected to explore the association of PVL-positive *S. aureus* with clinical disease, 4.9% were PVL positive and most were associated with skin and soft tissue infection especially abscesses.¹³ It has been reported that most of the PVL positive *S. aureus* causes significant infection in skin and in lower respiratory tract.¹ The cases of necrotizing pneumonia caused by PVL positive *S. aureus* have been reported from France, Sweden, the Netherlands and the UK.¹³ PVL gene has been detected in 11% in *S. aureus* causing pneumonia in the UK.¹³ PVL gene has also been detected in isolates responsible for community-acquired pneumonia, bacteremia, burn infections, and scalded skin syndrome.¹³ The occurrence of PVL positive *S. aureus* has been not been reported much in UTI. These reports suggest that PVL carriage depends largely on the geographical locations and the organisms that are present in a particular locality.

PVL positive *S. aureus* prevalence with respect to age

It has been stated that PVL carriage is associated with young age group, however Rossney et al. have stated that the difference in rates between isolates from adult and pediatric patients did not reach statistical significance.²² PVL positive *S. aureus* usually affect the healthy young children and young adults, and is generally associated with community acquired infections.²¹ Elderly people in hospitals who have weakened immune systems are the most likely to be infected by MRSA but PVL positive MRSA strains also affect young and healthy people. Since, the hospital strains can cause infection in any patient having the risk factor of acquiring the hospital acquired infection justifies the reported median age of occurrence of MRSA nosocomial infection as 70 years.²³

In an Australian study, in the age group 10 to 19 and 20 to 29, 50 % of the infections were caused by PVL positive *S. aureus* and there was a steady decline in the occurrence with the increase in age.²¹ The study was carried out in clinical isolates and the researchers have suggested that *S. aureus* were acquired by the children and young adult in the school and playing sports. The PVL positive *S. aureus* manifest skin and soft tissue infection, which are known to be easily transmitted. They have stated that the negative association of PVL with age is due to development of immunity with increase in age.²¹

PREVALENCE

As already stated, PVL has been reported to be produced by less than 2% of *S. aureus* in the UK, where the prevalence of PVL positive *S. aureus* had been reported to be low.¹³ The number of PVL positive infections in 2006 has increased more than twice the number identified in 2005.¹¹ It is not known whether the increase in number observed was due to actual increase in the infection rate or due to better case identification.¹¹ Reports from many countries have stated that the prevalence of PVL positive *S. aureus* has been in a range of 0.9% to 12.8%, whereas in MRSA PVL gene has been reported in 39%.²⁴ However, different PVL carriage rate have been reported from various regions. There are reports from the UK showing that the PVL carriage was observed only among MSSA (1.6 %) and not at all among MRSA.¹⁹ Occurrence of PVL positive *S. aureus* 2% has been reported in France.⁷ A comparatively high PVL *S. aureus* carriage rate 11.6% has been reported from Singapore in a tertiary public hospital.²⁵ In Korea 0.9% (5 isolates) of *S. aureus* isolates were PVL positive and of them one isolate was MRSA.²⁶

Table 2. PVL positive MSSA occurrence in various geographical regions.

Countries	Occurrence
Australia	16% ²¹
Singapore	11.6% ²⁵
France	2% ⁷
United Kingdom	1.6% ¹⁹
Korea (one was MRSA)	0.9% ²⁶

PVL carriage in relation to MRSA

PVL carriage among MRSA has been reported unusually dissimilar from various countries. Wannet has reported PVL gene carriage in 10% of the MRSA isolates from the Netherlands.²⁷ Whereas in Vienna PVL gene has been reported in 39% of MRSA and all PVL negative isolates were *mecA* negative.²⁴ Conversely presence of PVL gene among MRSA has been reported to be 97% in the USA.²⁸

Table 3. PVL positive MRSA occurrence in various geographical regions.

Countries	Occurrence
United States of America	97% ²⁸
Vienna	39% ²⁴
Netherland	10% ²⁷

MRSA that are resistant to ≥ 3 non β lactam antibiotics are termed as multi-resistant MRSA, mMRSA and those resistant to ≤ 2 non β lactam antibiotics as nonmulti-resistant MRSA, nmMRSA. Munckhof *et al.*, 2008, have reported that PVL carriage among mMRSA was 2% and was 57% among nmMRSA.²¹ Most of PVL positive isolates were health care associated. They have further reported that among MSSA the PVL carriage was 16%.²¹

COMMUNITY ACQUIRED INFECTION VERSUS HOSPITAL ACQUIRED INFECTION

It has been reported that the PVL producing *S. aureus* causing pneumonia are significantly community acquired.¹ Therefore, PVL genes have been identified as a stable marker of the community acquired MRSA strains worldwide.²⁹ However, there are reports on hospital acquired PVL positive *S. aureus* infections. Moreover, community acquired MRSA strains are frequently isolated from patients in hospital settings and these strains are transmitted among the patients in the hospitals.³⁰ Further, it has been stated that PVL is not the sole marker of community acquired MRSA infection, as all community acquired MRSA are not PVL carriers and PVL carriage also occur among the nosocomial MRSA isolates.²² In the UK, most infections have been reported to be associated with PVL positive MRSA. It has also been stated that the community-associated MRSA are more likely to produce PVL than hospital-associated MRSA.¹⁰ Supporting the fact

there are many reports stating that there is a higher occurrence of community acquired PVL positive isolates compared to hospital acquired isolates. Moreover, researchers have reported that these days PVL is found in a very high proportion of newly emerging community associated MRSA strains, with rates of 77% to 100%.^{31,32}

In a study carried out in Turkey in 2008, PVL gene was detected in 12 isolates (8 MRSA and 4 MSSA) among 262 MRSA and 43 MSSA. Of these 230 isolates were hospital acquired and 74 were community onset *S. aureus*.³³ It has been reported that of 25 isolates (1.8%, n=25/1389) positive for PVL in Ireland, 19 were community acquired MRSA and 6 were hospital acquired MRSA and there was significant difference between the PVL carriage rate among community acquired MRSA and hospital acquired MRSA.²² Moreover, in a study carried out in China it has been reported that the carriage rate of *S. aureus* was 12.8%. Of the isolates, 11.9 % were hospital acquired and 17.1 % were community acquired.³⁴

On the basis of above reports one can put forward that PVL carriage rate greater among community acquired MRSA compared to hospital acquired MRSA. It is not unusual as MRSA from community are becoming increasingly responsible for the nosocomial infections.³⁵ The virulent community acquired MRSA organisms may become established in the health care environment and become epidemic in nature.³⁵

MOLECULAR EPIDEMIOLOGY OF PVL POSITIVE MRSA

In hospital acquired infections clusters of MRSA are much more limited than for MSSA which is due to the acquisition of methicillin resistance genes by certain strains of the species.³⁶ These MRSA hospital isolates belonging to a particular restriction types cause infections whenever they came across suitable host. However, PVL Positive MRSA from community has also been reported to be phenotypically consistent.²⁴ There are many reports from the USA and Australia showing that the community acquired MRSA are moving into the hospitals, causing infection and are being transmitted among the hospitalized patients²¹ via health care workers.³⁷ Moreover, reports stating the prevalence of the *lukSF-PV* genes among the (37%) clinical MSSA isolates not significantly different from the prevalence of the *lukSF-PV* genes among the (31%) nasal carriage isolates (healthy population volunteers without hospital contact) in Auckland³⁸ pave way to the possibility that the organism from community possibly being responsible for the clinical infections. It has been stated that the community acquired MRSA is supplanting the traditional multi-resistant healthcare associated strains and has emerged as the most common cause of hospital acquired

infection in the USA.³⁹ Munckhof *et al.* have suggested that such a change in epidemiology is expected to occur globally and health care practitioners should be alert to this phenomenon.

LABORATORY DIAGNOSIS

***S. aureus* isolation:** Swabs or pus from the skin infections and sputum/aspirate from lower respiratory tracts are processed as described by American Society for Microbiology, ASM 2004.⁴⁰ Samples are inoculated onto blood agar and incubated for 18 hours at 37 °C for the isolation of *S. aureus*; and are identified. Antibiotic susceptibility of *S. aureus* isolate is done as recommended by Clinical Laboratory Standard Institute.⁴¹ For the identification of MRSA, Oxacillin or ceftoxitin disc diffusion, MRSA screen test (latex agglutination for the detection of altered penicillin binding protein), oxacillin screen agar test, determination of oxacillin minimum inhibitory concentration by microbroth dilution or E test are done.

PVL gene detection: All newly emerging community-acquired MRSA strains carry the PVL genes (present in a Gisland) and possess *mecA* gene. The *mecA* gene is present in a small mobile Gisland staphylococcal cassette chromosome *mec* (SCC*mec*) type IV or V genetic element which is easily transferred to other strains of *S. aureus*. This IV or V SCC*mec* is found only in CA-MRSA and not in hospital acquired MRSA. Hospital acquired MRSA possesses the large SCC*mec* type I to III.^{29, 42, 43}

Till few years back PCR was the only method for the detection of PVL genes.^{1,44} Later real time PCR was used for the detection of PVL gene.⁴⁵⁻⁷ Gene sequencing of PVL gene was then done by Nagakawa *et al* in 2005.⁴⁸ Recently multiplex PCR has been performed for the detection of PVL gene and *mecA* gene encoding the methicillin resistance in a single run. The new multiplex PCR assay targets the *Staphylococcus* genus-specific 16S rRNA gene that serves as an internal control. Multiplex PCR has the advantage that along with PVL gene MRSA isolate can be differentiated from the MSSA.⁴⁹

To demonstrate the genetic relatedness of community acquired MRSA isolates from hospital acquired MRSA clones circulating within defined geographic regions Multilocus sequence typing, MLST⁵⁰ and pulsotyping using pulsed-field gel electrophoresis, PFGE have been used.⁵¹

INFECTION CONTROL AND DECOLONIZATION

Since *S. aureus* resides as the commensal in nose, throat, perineum, axilla, skin lesions the patients should be screened for *S. aureus* carriage. The swab should be inoculated on the non selective media, blood agar to ensure recovery of the *S. aureus*. If found positive

the patient should be decolonized from skin or upper respiratory carriage.⁵² Decolonization is done by application of 2% mupirocin ointment three times daily for 5 days and chlorhexidine bath once daily for three days.⁵³ Screening (nose, throat, perineum, axilla, skin lesions) of close contacts for carriage of PVL positive *S. aureus* should be done and decolonized if found positive.⁵²

ANTIBIOTIC THERAPY

Infections caused by antibiotic susceptible strains of PVL positive *S. aureus* are successfully treated with derivatives of penicillin and erythromycin.⁵⁴ Isolates found in the UK have been reported to be sensitive to many antibiotics like tetracycline, ciprofloxacin, rifampicin, trimethoprim and fusidic acid.⁵⁴ PVL positive MRSA infections are difficult to treat as MRSA are generally multiresistant. Therefore, it has been suggested that PVL positive MRSA can be tackled with treatments that attack the bacteria on three fronts. The drugs must kill the bacteria, destroy their ability to make PVL toxins, and mop up the toxins already released.²

A wide range of potentially useful antibiotics are available for treatment. For the skin infections caused by PVL-positive *S. aureus* strains flucloxacillin, erythromycin and clindamycin; and combinations of doxycycline and rifampicin has been recommended.⁵⁴ Susceptibility of the isolates should be performed first.

For the treatment of necrotizing pneumonia several researchers have reported the value of combination therapy and have recommended combinations of vancomycin, clindamycin, linezolid, rifampicin and/or co-trimoxazole in high doses.^{17,18,55} Use of vancomycin alone has not been recommended.⁵⁶ Bactericidal action has been observed with intravenous flucloxacillin in combination with linezolid or rifampicin. While waiting for the antibiotic susceptibility linezolid can be used to cover MRSA. Clindamycin and linezolid have the added advantage of suppressing toxin production.⁵⁴

Adjunctive Treatment (Human intravenous polyclonal immunoglobulin) - Intravenous Immunoglobulin (IVIG)

In addition to intensive care support and high dose antibiotic therapy, intravenous immunoglobulin (IVIG) should be considered because of high mortality.¹⁷ The dosage of 2 g/kg of IVIG (as recommended in streptococcal toxic shock syndrome) may be useful for PVL-positive *S. aureus* infections.⁵⁶⁻⁸ The therapy has been included in successful therapy regimens in the treatment of MRSA/MSSA severe sepsis.⁶ Justification for IVIG use cannot be directly attributed only to the presence of anti-PVL antibodies, since IVIG has been

shown to bind to recombinant LukS-PV and LukF-PV components.⁵⁹ Moreover, a commercial IVIG preparation has shown to neutralize pore formation in PMNs and the cytopathic effect of recombinant PVL. There are reports suggesting that immunization with purified PVL components conferred some protection against PVL-induced dermonecrosis.^{60,61} These data suggest that further studies are needed to determine the usefulness of anti-PVL antibody in therapy or a PVL based vaccine in disease prevention.

CONCLUSIONS

The virulent, PVL positive, Community acquired MRSA isolates belonging to different *S. aureus* genetic lineages have been emerging around the world. These organisms are beginning to encroach on healthcare environments where they form a stable population and could become endemic. MRSA if become multiresistant in the hospital environment due to antibiotic selection pressure or acquisition of resistance gene *mecA*, the situation can be deleterious. Therefore every isolate of *S. aureus* should be considered as potential to be PVL positive, carefully monitored, molecular epidemiology performed and tackled before they become a rife in the hospital as well as in the community.

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