# Chasing the Link Between Panton-Valentine Leukocidin and CA-MRSA

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## Abstract

DNA from 86 natural nasal isolates, previously identified as methicillin-resistant staphylococci (aureus and non-aureus) by 16srRNA sequencing, were assayed for the presence of the phage encoded Panton-Valentine Leukocidin (PVL) gene. The PVL gene is carried by one of 8 or 9 lysogenic phage known to infect and insert into the SCCmec (Staphylococcus Chromosomal Cassett mec) of methicillin-resistant Staphylococcus aureus. SCCmec is a highly mobile pathogenicity island, that is transferred between different species of staphylococci during promiscuous conjugation.

For epidemiological purposes, PVL is thought to be a molecular marker of community-acquired methicillin-resistant Staphylococcus aureus (CA-MRS) versus hospital acquired methicillin-resistant Staphylococcus aureus (HA-MRS). This designation may have merit given the increasing prevalence of methicillin-resistant staphylococci (MRS) that are PVL+ but not (yet) implicated in overt disease. In the study we show that 85 out of 86 natural isolates, obtained from a population of healthy carriers, possess the PVL gene. In addition, 20 of the PVL MRS are non-aureus that are not endemic to humans. Thus, this study provides may provide evidence strengthening the link between PVL and CA-MRS.

## Introduction

Staphylococcus (aureus and non-aureus) are commonly found on normal skin and mucosal surfaces with both considered to be opportunistic pathogens. In the past staphylococci infections were treated with penicillin, but with increased selective pressure came an unintended consequence, bacterial resistance which in turn gave rise to Methicillin Resistant Staphylococcus Aureus(MRSA) and Methicillin Resistant Staphylococci (MRS) [1]. Both MRSA and MRS infections can be the result of a recent hospitalization or surgery, dialysis, indwelling percutaneous medical device and catheter, residence in a long-term care facility, or more recently among healthy community-dwelling persons [2]. Recent genomic sequence of a CA-MRS isolate indicated the presence not only of a novel smaller variant of the methicillin-resistance locus (SCCmec), but also that of the locus for the Panton-Valentine leukocidin (PVL) [2]. PVL was connected with soft tissue infection in 1932 by Sir Philip Noel Panton and Francis Valentine [3]. It is a cytotoxin with that correlates with higher virulence in Staphylococcus aureus. The PVL gene can be found in the Φ-PVL bacteriophage and has been incorporated into many different types of staphylococcal bacteria [4]. Panton-Valentine leukocidin is part of a family of synergohymenotropic toxins. The PVL gene in S. aureus codes for two co-transcribed secreted proteins: LukS-PV and LukF-PV. These two proteins are able to work together to attack host defense cells and creates pores in host membrane [5]. PVL is a beta-pore-forming toxin similar to alpha-hemolysin [6],[7]. It is able to induce a major histamine response from basophilic granulocytes and mast cells. LukS-PV and LukF-PV attack granulocytes and neutrophils, causing them to release high levels of enzymes such as lysozyme, B-glucuronidase, interleukin-8, and oxygen metabolites [8]. The increased levels of these enzymes and metabolites cause a rise in toxicity and eventual death of host leukocytes. MRS containing the PVL gene has been associated with dermal necrosis in rabbits [9] and virulent necrotizing pneumonia in humans [10]. Its believed that the PVL toxin is injected into target leukocytes via a type III secretion system. A type III secretion system Resembles a molecular syringe, and form channels that cross the host cell membrane, which enable MRS to inject the PVL toxin into the host cell cytoplasm [11]. Outside studies have show an absence of PVL among MRS isolates from hospital environment which indicates that PVL has poor association with hospital acquired MRS. This indicates that with all likelihood, PVL can be used as a genetic marker to CA-MRS.

## Research Objectives

In this study we examine 86 natural methicillin resistant staphylococci, isolated from healthy carriers, in order to see if they contain the gene coding for Panton-Valentine Leukocidin. We hope information on these isolates is able to provide further information on the link between PVL and community acquired methicillin resistant staphylococci.

## Methods

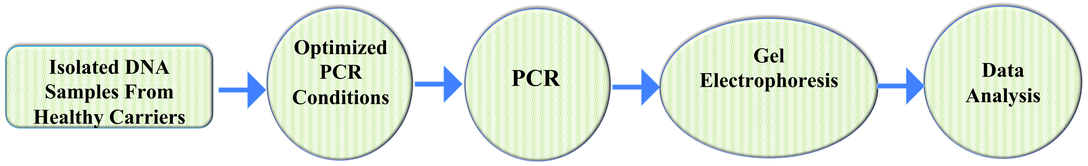


**Figure 1: Methods flow chart**

## Data Analysis and Results

Nasal isolates taken from approximately 86 healthy individuals over a five year period were isolate and used for the study. Through PCR Amplification and Gel Electrophoresis, we can see that 85 of the 86 individuals contain the 83 base pair PVL gene.

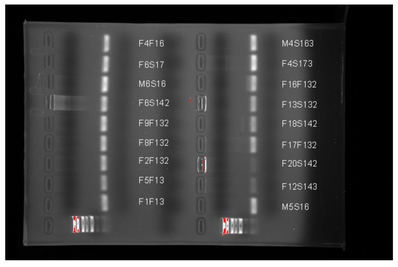


**Figure 1.** Gel A, the above strains of MRS all contain the PVL gene

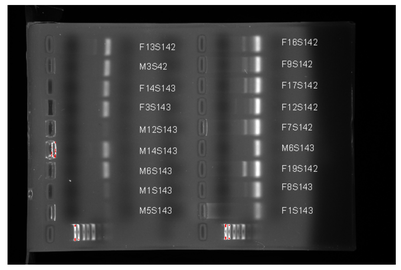


**Figure 2.** Gel B, the above strains of MRS all contain the PVL gene

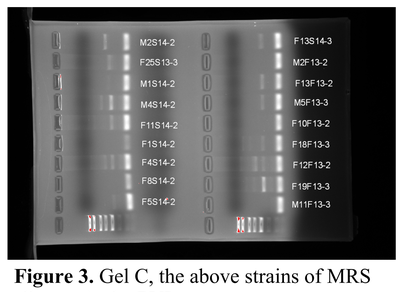


**Figure 3.** Gel C, the above strains of MRS all contain the PVL gene

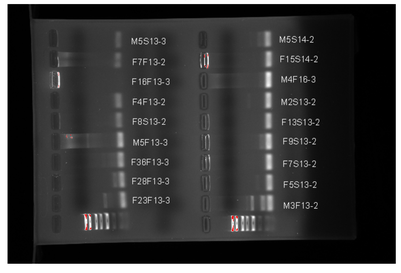


**Figure 4.** Gel D, 17 of the 18 MRS strains are PVL positive. One of the strains is PVL negative.

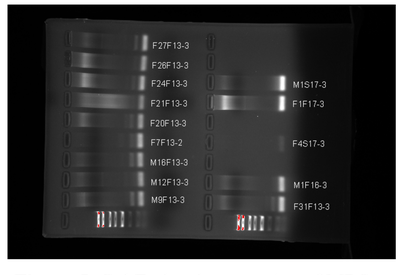


**Figure 5.** Gel E, the above strains of MRS all contain the PVL gene

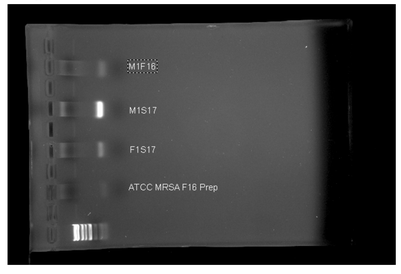


**Figure 6**. ATCC 43300 was used as a PVL positive control throughout the experiment.

## Discussion

In this study we examined 86 natural isolates of MRS to see if they contained the gene for the PVL toxin. Of the 86 isolates examined, 85 possessed the PVL gene. 20 of the isolate strains were non-aureus MRS which are not endemic towards humans, indicating this toxin is still present in strains affecting other species. PVL is found in less than 5% of Staphylococcus aureus strains, but it is found in a high percentage of strains that are associated with tissue necrosis and leukocyte death [12]. Current evidence suggest that the PVL gene is normally present in CA-MRS and is not usually an element of any hospital acquired infection [13]. The natural isolates used in the lab were community acquired. Our investigation further demonstrates the high presence of PVL in CA-MRS, indicating a strong relationship between staphylococci and the toxin. The association of CA-MRS and PVL gives us more information about the severity of CA-MRS and possible symptoms that can be caused by it. Future studies may be able to investigate the presence of PVL in HA-MRS further to determine if PVL is a truly able to act as a marker for CA-MRS.

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