Do Differences in Panton-Valentine Leukocidin Production among International Methicillin-Resistant *Staphylococcus aureus* Clones Affect Disease Presentation and Severity?

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Panton-Valentine leukocidin (PVL) production by methicillin-resistant *Staphylococcus aureus* (MRSA) was determined *in vitro* using the enzyme-linked immunosorbent assay (ELISA), and associations with clinical presentation and bacterial genetic characteristics were examined. PVL production ranged from 0.02 to 4.865 μg/ml and correlated with a multilocus sequence type (MLST) clonal complex associated with specific PVL phage types. A relationship between PVL production and clinical presentation or patient demographics could not be demonstrated.

Panton-Valentine leukocidin (PVL) is a bicomponent leukotoxin that can be produced by *Staphylococcus aureus*, including many community-associated strains of methicillin-resistant *S. aureus* (CA-MRSA). The PVL-encoding genes (*lukS-PV* and *lukF-PV*) reside in the genomes of several icosahedral or elongated head-shaped temperate bacteriophages (9, 33). Clinically, MRSA which harbor PVL (PVL-MRSA) are most often associated with pyogenic skin and soft tissue infections (SSTI) (31) but can also cause life-threatening disease, most notably necrotizing pneumonia (25). Although the role of PVL as a virulence determinant has been questioned (7, 8, 14, 43), some animal-model-based investigations have demonstrated its pathogenicity (10, 13, 15, 29, 32, 42), and a clear epidemiological association is apparent between PVL and successful lineages of CA-MRSA (23; M. J. Ellington, C. Perry, M. Ganner, M. Warner, I. McCormick-Smith, R. Hill, L. Shallcross, S. Sabersheik, A. Holmes, and A. Kearns, unpublished data). In Europe, multilocus sequence type (MLST) ST80-MRSA-SCCmeCIV (European clone) predominates, but other clones, such as ST8-SCCmeCIVA (USA300) and ST30-SCCmeCIV (south-west Pacific clone), are notable. In England and Wales, additional PVL-MRSA MLST clonal complexes (CCs), namely, CC5, CC19, CC22, CC59, and CC88, and ST93 clones have also been reported (19, 21). Minor sequence variation in the PVL genes correlates with the PVL bacteriophage (3, 36), and bacteriophages are known to have limited host ranges with respect to *S. aureus* strains and MLST lineage (3).

Previous work has shown intra- and interstrain variation in PVL production *in vitro*. Interstrain variability has been previously reported for ST8 (2, 26) and ST80 and ST93 (2); ST8 PVL-positive strains corresponding to the USA300 clone have been shown to be strong PVL producers *in vitro*, while ST80 (European clone) strains produce 7-fold less PVL (2).

Set against this background of heterogeneity among PVL-MRSA, this study was designed to examine PVL production among diverse international lineages of PVL-MRSA identified in England and Wales and to investigate any relationships with basic bacterial genetic characteristics, including MLST CCs, PVL-encoding phages, SCCmec type (larger SCCmec clones have been shown to affect bacterial fitness [30]), and agr type (linked to the expression of virulence factors [5, 45]). Moreover, the relationship between isolates and clinical disease presentation is complex and likely to be dependent on a multiplicity of host factors. While it has been suggested previously that PVL production does not influence disease presentation in patients (2), this work was also designed to investigate whether the level of PVL production may be related to clinical presentation and to analyze patient demographics and assess epidemiological relationships with respect to clinical disease.

The *Staphylococcus Reference Unit* (SRU) for England and Wales receives isolates from a wide spectrum of disease presentations for surveillance and outbreak investigation purposes. In this work, 142 isolates were studied; these had been referred to the SRU between 2005 and 2008 (from a total prevalence of 1,477 PVL-MRSA) from centers across the nine regions of England designated by the Health Protection Agency (http://www.hpa.org.uk/HPA/ProductsServices /InfectiousDiseases/RegionalMicrobiologyNetwork/) and from Wales (defined as a 10th region for the purposes of this study) and were selected to maximize demographic, phenotypic, and genotypic diversity. The study isolates were selected to represent the main lineages of PVL-MRSA occurring nationally; all had been characterized previously by MLST, pulsed-field gel electrophoresis (PFGE), SCCmec, spa, and arginine-catabolic mobile element (ACME) PCR as belonging to MLST CCs 1, 5, 8, 22, 30, 59, 80, and 88 and ST93 (4, 19–21, 22). Where possible, isolates were also selected to include a range of disease presentations, which were categorized into six groups: community-acquired pneumonia (CAP), bacteremia (Bact), SSTI, upper respiratory tract infection (URTI), asymptomatic carriage (AS), and not known (NK).
Eight PCR s were performed to detect five of the PVL-encoding phages (ΦSa2958, ΦSa2MW, ΦPV1, Φ108PV1, and ΦSLT), as described previously (4, 34). A PCR (fragment size, 680 bp) was designed to detect a sixth PVL phage, ΦSa2USA, using methods and primers described by Boakes et al. (3) (the more recently described Φtp310-1 was not examined [46]). Study isolates were cultured in triplicate in casein hydrolysate-yeast (CCY) medium, and PVL was detected and quantified using an antibody sandwich enzyme-linked immunosorbent assay (ELISA) targeting LukS-PV as described by Badiou et al. (2). The mean PVL production from triplicate cultures was taken for each isolate.

The Kruskal-Wallis equality-of-populations rank test was used as an initial test for variation in PVL production between isolates. A generalized linear model (GLM) was used to examine how PVL production was affected by a number of different fixed, specific variables, including bacterial lineage (MLST CC), type of PVL-encoding phage, and SCCmec and agr type. Possible interaction between these variables was also explored. In addition, the effect of PVL production on the disease presentation was examined. The data were normalized by transforming the PVL production data by log (PVL values × 100) (to allow for low values in the data set).

A minimally adequate model was derived in order to best describe the relationship between the variables and PVL production. Categorical scatter plots showing the mean and 95% confidence intervals were used to identify differences in PVL titers across different variables. The level of statistical significance was set at a P of <0.05. Contingency tables were created, and Pearson’s chi-square tests were used to examine the significance of age, sex of the patient, and geographic region on clinical disease presentation. Statistical analysis and plotting were performed in R (40).

This work shows a hitherto-unrecognized variability in the production of PVL toxin across internationally disseminated lineages of PVL-MRSA that have emerged recently. Using ELISA, we determined that the PVL-MRSA tested (chosen to be representative of genetically diverse strains identified in England and Wales [n = 142]) produced 0.02 to 4.865 μg/ml PVL (mean, 0.5 μg/ml) (see Table S1 in the supplemental material).

Furthermore, 94% (n = 134) of the study isolates produced enough PVL in vitro to induce human polymorphonuclear leukocyte activation (≥0.05 μg/ml) (28) and 60% (n = 85) produced PVL at concentrations toxic for human leukocytes (≥0.3 μg [24]). These data support previous observations that PVL may induce a host inflammatory response during infection (1, 2).

Patient demographics and clinical presentations for the 142 individuals were typical for PVL-MRSA (27), and cases were distributed widely throughout England and Wales (see Table S1 in the supplemental material). Epidemiological data show that PVL-positive S. aureus strains are associated with some severe forms of skin infection (12, 31, 37, 47), bone and joint infection (3, 9), and necrotizing pneumonia (12, 13). For these infections, experimental data support the role of PVL in disease severity (6, 10, 13, 41).

In summary, this work shows that PVL production in MRSA is variable, with significant association with MLST CC. Specificities of PVL-encoding phages for MLST CCs were also apparent (3). PVL production was not affected by SCCmec or agr type, although the effect of agr expression on PVL production warrants further investigation. While further work probing the relationship between PVL production and disease outcome is necessary, this work suggests that there is no statistical relationship between PVL and the most severe clinical presentations of PVL-MRSA infection. In view of the complex nature of pathogenicity, adapting therapeutic strategies in accordance with current guidance (38) may ameliorate disease severity.

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FIG 1 Categorical scatter plots of mean PVL concentrations (μg/ml) produced by PVL-MRSA from different disease presentations (a), MLST CCs (b), and PVL-encoding phages (c). AS, asymptomatic carriage; CAP, community-acquired pneumonia; SSTI, skin and soft tissue infection; URTI, upper respiratory tract infection; Bact, bacteremia; NK, not known. The number of cases for each disease presentation is shown (n). Black dots indicate means, and the error bars show the 95% confidence intervals surrounding the means. PVL production was measured in μg/ml. The isolate producing 4.86 μg/ml was considered an outlier and is not shown.

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REFERENCES