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.

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, ΦPVl, Φ108PVl, 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 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). However, in this study, there was no correlation between clinical presentation and high levels of PVL production observed in vitro (P > 0.05) (Fig. 1a), which may be attributable to the heterogenous nature of the infections and patients. Age, sex, and geographic region did not have a significant effect on disease presentation (P > 0.05) (see Table S2 in the supplemental material). For those infected with PVL-MRSA, it is probable that bacterial and host factors, such as bacterial load, body site, nature of the infection, history of recurrent disease, therapeutic strategies, underlying comorbidities, and immune status, influence the level of PVL produced during the course of infection (11). Due to the complexity of virulence and the multifactorial nature of pathogenicity in PVL-MRSA infection, it is likely that no single bacterial virulence factor is responsible for causing severe infection. Whether PVL is a marker for disease severity or not, it seems likely that antibiotic combinations that inhibit both bacterial replication and the release of virulence factors, including PVL, may improve the outcome of severe infections caused by PVL-producing S. aureus strains (in vitro, β-lactam agents increase PVL release, while protein synthesis inhibitors, such as clindamycin, rifampin, and lincomycin, suppress it [16, 17]).

These data support previous evidence that within MLST lineages, isolates harbor different PVL phages (3) and produce variable amounts of PVL (see Table S1 in the supplemental material and Fig. 1b and c). Little variation was observed within the replicates of each isolate (standard deviation [SD] range, 0.062 to 0.206) but intralineage variation in PVL production did occur, evidenced by the broad differences in standard deviations shown in Table S1 (e.g., CC8). Using the Kruskal-Wallis equality-of-populations rank test, a marked difference in PVL production across the study isolates was apparent (P < 0.001). Significant variation between the lineages of PVL-MRSA was identified; CC8 with ΦSa2USA produced the most PVL, significantly more than CCs 5, 22, 80, and 88 (P value < 0.001) (Tables S1 and S3 and Fig. 1b). CC88 with an unknown elongated PVL phage produced the smallest amount of PVL, significantly less than all other lineages excepting CC80 (P < 0.05) (Tables S1 and S3 and Fig. 1b). The GLM showed a significant relationship between PVL production and MLST (CC) (P < 0.001) (Table S3). Previous work has shown that the insertion of PVL-encoding phage is lineage specific (3, 45) and that expression of the PVL genes is dependent on phage lifestyle (45) and host chromosomal regulatory networks; notably, agr and saeRS are linked to the pathogenesis of CA-MRSA (5, 35, 44, 45). This study shows that agr type (or SCCmec type) did not significantly affect PVL production; however, differences attributable to variable agr expression cannot be ruled out (39).

It is important to acknowledge that these in vitro observations may not be representative of PVL production in vivo (2). Some parallels may exist, however, as recent work has shown that PVL production in vivo is increased by β-lactam treatment (18). Reliable comparison of in vitro and in vivo findings would entail quantitative cultures of clinical material to estimate bacterial loads, which is difficult to correlate (26) and will likely be further complicated by difficulties in correlating PVL level with clinical disease, due to the complex and obscuring effect of host factors.

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 production 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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