Coexistence of Panton-Valentine Leukocidin–Positive and –Negative Community-Associated Methicillin-Resistant *Staphylococcus aureus* USA400 Sibling Strains in a Large Canadian Health-Care Region

Kunyan Zhang,1,2,3,4,5,6 Jo-Ann McClure,7 Sameer Elsayed,3,5 Jonathan Tan,7 and John M. Conly1,2,3,4,5,6

1Centre for Antimicrobial Resistance, Calgary Health Region/Calgary Laboratory Services/University of Calgary, Departments of 2Pathology and Laboratory Medicine, 3Microbiology and Infectious Diseases, and 4Medicine, and 5Institute of Infection, Immunity and Inflammation, University of Calgary, and 6Calgary Laboratory Services, Calgary, Alberta, Canada

(See the editorial commentary by Gorwitz, on pages 179–82.)

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains often carry the Panton-Valentine leukocidin (PVL) genes. However, the specific role that PVL plays in the epidemiological features and pathogenesis of CA-MRSA infections has remained undefined and controversial. Conducting a retrospective study on a natural population of MRSA clinical isolates recovered from community and hospital patients in a large Canadian health-care region during a 6-year period, we identified the coexistence of 2 USA400 (a major clonal group of CA-MRSA) sibling strains with and without PVL genes. Polymerase chain reaction and sequence analysis indicated that the PVL-carrying prophage $\phi$Sa2mw was present in PVL+/H11545 but absent in PVL−/H11546 USA400 isolates. These strains shared identical genotypic and phenotypic properties and similar clinical characteristics. This study provides direct evidence that PVL genes are not necessarily the key determinants associated with the increasing dissemination of CA-MRSA strains, suggesting that the genomic milieu may play a greater role in this regard.

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be a major cause of hospital-associated infections. Recently, strains of MRSA have been recovered from infections in community settings; they are referred to as “community-associated MRSA” (CA-MRSA) [1]. These newly emerging CA-MRSA strains are causing serious community-acquired infections in otherwise healthy children, athletes, and other individuals lacking typical risk factors for nosocomial MRSA acquisition [2, 3]. Although diversity and variation in their genomic backgrounds and antibiogram patterns exist, these newly emerging CA-MRSA strains often carry the Panton-Valentine leukocidin (PVL) virulence genes and a recently described small mobile staphylococcal cassette chromosome mec (SCCmec) type IV or V genetic element, in contrast to larger SCCmec types (I–III) that are prevalent in hospital-associated MRSA (HA-MRSA) [1, 4].

The USA400 strain is a major clonal group of CA-MRSA currently circulating in the United States and Canada and one that has been implicated in outbreaks of community-onset infections associated with serious morbidity and mortality [5–7].

The Calgary Health Region (CHR) is one of Canada’s largest integrated urban health-care regions and provides medical services for a population of ~1.25 million people, with 1900 acute care bed housed in 4 acute-care facilities and several community health centers in the Calgary area, including the cities of Calgary and Airdrie and ~20 nearby, smaller outlying communities. We conducted a retrospective study of a natural population
of MRSA clinical isolates recovered from patients in the CHR during a 6-year period and identified the coexistence of 2 USA400 sibling strains differing with respect to carriage of PVL genes but sharing identical genotypic and phenotypic properties and similar clinical characteristics.

PATIENTS, MATERIALS, AND METHODS

Bacterial strains and isolates. Calgary Laboratory Services (CLS) provides centralized diagnostic microbiology services for the entire CHR. All isolates of MRSA recovered at CLS from correspondingly infected and/or colonized patients in the CHR during a 6-year period from 1 January 2000 to 31 December 2005 were included in this study. The Canadian major epidemic MRSA reference strains CMRSA1–CMRSA10 (including the reference strain of USA400, known as “MRSA7” in Canada) were provided by the National Microbiology Laboratory, Health Canada (Winnipeg, Manitoba) [7, 8].

Identification and phenotypic susceptibility testing of staphylococcal isolates. The staphylococcal isolates were identified morphologically and biochemically by standard laboratory procedures. The coagulase plasma test (Remel) was performed on organisms exhibiting typical staphylococcal colony morphology, to allow S. aureus to be differentiated from coagulase-negative staphylococci. Screening for methicillin and other antibiotic-resistant phenotypes was performed by use of VITEK 1 (bioMerieux) and the Clinical and Laboratory Standards Institute oxacillin agar screen, whereas confirmation of methicillin resistance was achieved by use of an in-house polymerase chain reaction (PCR) assay for the mecA gene [9].

Case definition, clinical data, and statistical analyses. This primarily laboratory-based surveillance study identified new cases of MRSA as occurring in those patients who had had no previous MRSA infection/colonization during the preceding year. Isolates were categorized according to anatomic site of isolation—skin/wound, nares, and blood/sterile body fluid sources. MRSA isolates were classified as being CA-MRSA when recovered from a specimen collected from a patient in the community. For patients who attended hospitals, we used criteria from the Canadian Nosocomial Infection Surveillance Program to identify individuals with MRSA infection/colonization that was potentially hospital acquired (i.e., individuals with infection developing >3 days after admission to hospital and/or with a history of hospitalization or residence in a long-term care facility during the 12 months preceding the onset of symptoms) [10]. Clinical data were collected via chart review of medical records from hospitalized patients and were classified into 3 categories—namely, colonization, mild infections, severe infections—on the basis of previously published criteria (i.e., severe infections were considered to be abscess, ulcer, cellulitis, bursitis, septic arthritis, and pneumonia, whereas furuncles, impetigo, and other nonsevere infections were considered to be minor infections) [11]. The chart review was conducted according to the guidelines and regulations of the Conjoint Health Research Ethics Board of the University of Calgary and the CHR. Statistical analyses were performed by use of GraphPad InStat and PRISM (version 3.0 for Windows; GraphPad Software). For analysis of contingency data derived from 2 groups, Fisher’s exact test was used. For comparisons of group rate trends, the χ² test with Yate’s correction for trend was performed. P < .05 was considered to be significant.

Molecular and genomic characterization of isolates. All new MRSA cases identified in the CHR during the period from 1 January 2000 to 31 December 2005 were DNA fingerprinted by pulsed-field gel electrophoresis (PFGE) after being digested by use of Smal, according to a standardized protocol [8]. PFGE-generated DNA fingerprints were digitized and analyzed by use of BioNumerics (version 3.5; Applied Maths) software using a position tolerance of 1.0 and an optimization of 1.0. Isolates with a PFGE pattern identical to that of the USA400 strain were tested for PVL genes by a PCR assay [12] and were typed for SCCmec by a multiplex PCR assay, to classify the isolates as type and subtypes I–III, IVa–IVd, and V [13]. Representative isolates were further characterized by multilocus sequence typing (MLST) [14], staphylococcal protein A (spa) typing [15], and accessory gene regulator (agr) typing [16], as described elsewhere. The identification of MRSA isolates as being USA400 CA-MRSA strains was based on the similarity of PFGE patterns to those of the USA400 control strains (on the basis of previously published criteria [17]), SCCmec type IVa, spa type t128, MLST ST1, and agr type III. Profiles based on 25 virulence genes comprising 11 toxin genes, 11 adhesin genes, and 3 exoenzyme genes were determined by PCR amplification using previously described primer pairs, as outlined in table 1.

Sequence alignment and strain- and phage-specific primer design. New sets of specific primers for USA400 CA-MRSA strains and for prophage φSa2mw, which carries PVL genes in USA400 strains, were designed on the basis of a comprehensive analysis and alignment of the Staphylococcus-species genomes currently available in the GenBank database (National Center for Biotechnology Information; information updated as of July 2006) and obtained from a manuscript [28]. The gene targets and specificity of each primer pair were as follows: USA400 strain-specific primers MW756-F (5’-TGGTTAGCTATGAA TGTAGTTGC-3’) and MW756-R (5’-GTCCATCCTCTGTA- AATTTCGC-3’), which target the MW756 gene (which codes for a hypothetical protein) on genomic island ϕSa3 of the USA400 MW2 strain; φSa2mw prophage-specific primers phi-int-F4 (5’- CAAATTTGAAATTTTCGC-3’) and phi-int-R4 (5’- TCCAGGATTAAAGAGG-3’), which target the MW1409 gene (which codes for a hypothetical protein) on prophage φSa2mw of the USA400 MW2 strain; and the second set of φSa2mw prophage-specific primers, phi-F (5’- GAAAAAGTATA CGGACTG-3’) and phi-R (5’- ATATTGCTCCTGGA- TACTGC-3’), which target the MW1438 gene (which codes for a
Table 1. Toxin, adhesin, and exoenzyme virulence-gene profiles of Panton-Valentine leukocidin (PVL)* and PVL− USA400 strains of methicillin-resistant *Staphylococcus aureus* (MRSA).

<table>
<thead>
<tr>
<th>PFGE profile</th>
<th>Isolate</th>
<th>SCC mec type</th>
<th>agr type</th>
<th>spa type</th>
<th>MLST type</th>
<th>Toxin genes</th>
<th>Adhesin genes</th>
<th>Exoenzyme genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVL− USA400 (B)</td>
<td>7</td>
<td>N/a</td>
<td>III</td>
<td>t128</td>
<td>ST1</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>PVL− USA400 (B)</td>
<td>1</td>
<td>N/a</td>
<td>III</td>
<td>Unnamed</td>
<td>ST1</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PVL− USA400 (A)</td>
<td>85</td>
<td>N/a</td>
<td>III</td>
<td>t128</td>
<td>ST1</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>PVL− USA400 (A)</td>
<td>26</td>
<td>N/a</td>
<td>III</td>
<td>t128</td>
<td>ST1</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CMRSA-7</td>
<td>1</td>
<td>N/a</td>
<td>III</td>
<td>t128</td>
<td>ST1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE. The presence or absence of 25 virulence genes was determined by polymerase chain reaction (PCR) amplification using previously described primer pairs from the references indicated in parentheses in the sentences below. Toxin genes’ designations (sources) are *sea/seb/sec/sed/see/sej*, staphylococcal enterotoxin A/B/C/D/E/H/I/J (sea [18], seb/sec/see [19], sed/sej [20], and see [21]); *tst*, toxic shock–syndrome toxin ([19]); and *hlg*, gamma toxin ([22]). Adhesin genes’ designations (sources) are *fnbA*, fibronectin adhesive-molecule A ([23]); *fnbB*, fibronectin adhesive-molecule B ([24]); *cnaA/B*, collagen adhesive-molecule A/B ([25]); *sdrC/sdrD/sdrE*, putative adhesin ([16]); *ebp*, bone sialoprotein adhesin ([16]); *map/eap*, major-histocompatibility-complex class II–analogue protein ([16]); and *clfA*, clumping factor ([26]). Exoenzyme genes’ designations (sources) are *ica*, polysaccharide intercellular adhesin ([16]); *coa*, coagulase ([27]); and *V8*, serine protease ([16]). A plus sign (+) denotes that the strain is positive for the tested gene; a minus sign (−) denotes the strain is negative for the tested gene. agr, accessory gene regulator; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; PVL, Panton-Valentine leukocidin; SCC mec, staphylococcal cassette chromosome mec; spa, staphylococcal protein A gene (unnamed spa-type motif, UJJJJJFE).
hypothetical protein) on prophage φSa2mw of the USA400 MW2 strain. The second set of primers (i.e., that which targets the MW1438 gene) is not exclusively complementary to φSa2mw phage, because hybridization to other PVL-carrying phages, such as φSaLT [29] and φ108PVL [30], may also occur. PCR was performed in a 25-μL reaction mixture containing 2 μL of template DNA prepared as described elsewhere [9], 50 mmol/L KCl, 20 mmol/L Tris-HCl (pH 8.4), 2.5 mmol/L MgCl2, 0.2 mmol/L each dNTP (i.e., dATP, dTTP, dGTP, and dCTP) (Invitrogen), 0.2 μmol/L each primer, and 1.0 unit of Taq DNA polymerase (Invitrogen); the thermocycling conditions were 4 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C and ending with a final extension step for 10 min at 72°C [28].

RESULTS

Identical genotypic and phenotypic characteristics of PVL+/- USA400 isolates. During the 6-year period from January 2000 to December 2005, a total of 121 isolates were found to have PFGE patterns identical or similar to those of the USA400 reference strain, CMRSA7. Three patterns, A–C, were identified (figure 1). Pattern A, which was indistinguishable from USA400 control strain CMRSA7 (which is the same as the PVL+ USA400 MW2 strain [7]), was identified in 111 (91.7%) isolates; a pattern B profile was shared by 8 (6.6%); a pattern C profile was shared by 2 (1.7%). However, all of these isolates carried the SCCmec type IVa element and shared the same MLST ST1 profile (1-1-1-1-1-1-1), spa type t037 motif (UJJJJJFE) (figure 1). All isolates were resistant to β-lactams but were uniformly susceptible to all other antibiotics except erythromycin (67.8% of isolates were resistant) and tetracycline (3.3% of isolates were resistant) (figure 1).

Epidemiological features of PVL+/- USA400 isolates. Despite sharing identical/similar PFGE patterns and identical genotypic and phenotypic characteristics, only 27 (22.3%) of 121 isolates were determined to be PVL+, whereas the remaining 94 (77.7%) were PVL−. Interestingly, the yearly proportion of MRSA isolates that were USA400 remained quite stable (4.2%, 6.0%, 4.5%, 7.6%, and 6.0%, during the years 2000, 2002, 2003, 2004, and 2005, respectively) except for the year 2001 (when this proportion was 19.2%), although the number of newly diagnosed cases of MRSA infection/colonization in the CHR gradually increased on a yearly basis, with 71, 130, 167, 200, 355, and 770 new cases being identified during the years 2000, 2001, 2002, 2003, 2004, and 2005, respectively) except for the year 2001 (when this proportion was 19.2%), although the number of newly diagnosed cases of MRSA infection/colonization in the CHR gradually increased on a yearly basis, with 71, 130, 167, 200, 355, and 770 new cases being identified during the years 2000, 2001, 2002, 2003, 2004, and 2005, respectively) except for the year 2001 (when this proportion was 19.2%), although the number of newly diagnosed cases of MRSA infection/colonization in the CHR gradually increased on a yearly basis, with 71, 130, 167, 200, 355, and 770 new cases being identified during the years 2000, 2001, 2002, 2003, 2004, and 2005, respectively) except for the year 2001 (when this proportion was 19.2%), although the number of newly diagnosed cases of MRSA infection/colonization in the CHR gradually increased on a yearly basis, with 71, 130, 167, 200, 355, and 770 new cases being identified during the years 2000, 2001, 2002, 2003, 2004, and 2005, respectively) except for the year 2001 (when this proportion was 19.2%), although the number of newly diagnosed cases of MRSA infection/colonization in the CHR gradually increased on a yearly basis, with 71, 130, 167, 200, 355, and 770 new cases being identified during the years 2000, 2001, 2002, 2003, 2004, and 2005, respectively).
Figure 2. Epidemiological features of Panton-Valentine leukocidin (PVL)\(^{+/-}\) and PVL\(^{-}\) USA400 strains. A, Gradual increase in number of newly diagnosed cases of methicillin-resistant Staphylococcus aureus (MRSA) infection/colonization in the Calgary Health Region, showing stable yearly proportion of USA400 isolates after 2001. B, Varying yearly number of USA400 isolates, with stable proportion of PVL\(^{+/-}\) strains and PVL\(^{-}\) predominance after 2000. C, USA400 strains more often associated with MRSA infections identified in the community (CA-MRSA) than in the hospital (HA-MRSA) \((P < .0001)\) but with similar PVL carriage rates \((P = .615)\). D, USA400 strains more often associated with skin/wound infections than with infections of other body sites (e.g., blood/sterile fluids and nares) \((P < .0001)\) but with similar PVL carriage rates in isolates from different sources \((P = .5981)\).

(76.0% CA-MRSA vs. 24.0% HA-MRSA; relative risk, 3.17 [95% confidence interval [CI], 2.27–4.43] \([P < .0001, by Fisher’s exact test]\), but there was no difference in terms of PVL-carriage rates (23.9% PVL\(^{+}\) in CA-MRSA vs. 17.2% PVL\(^{+}\) in HA-MRSA \([P = .6105, by Fisher’s exact test]\)) (figure 2C). Moreover, these strains were more often associated with skin/wound infections than with infections at other body sites (8.3% blood/sterile fluids, 8.3% nares, and 83.5% skin/wound \([P < .0001, by \chi^2 test]\), but the infection-site frequency of PVL\(^{+}\) isolates was relatively similar (20% blood/sterile fluids, 10% nares, and 23.8% skin/wound \([P = .5981, by \chi^2 test]\)) (figure 2D).

Clinical features of PVL\(^{+/-}\) USA400 colonized/infected patients. To determine whether unique clinical features were associated with infections caused by PVL\(^{+/-}\) USA400 strains, we conducted orderly chart reviews of medical records of hospital patients infected with these USA400 strains. Complete medical records were available for 42 inpatients, 19 of whom were found to have CA-MRSA infection and 23 of whom were found to have HA-MRSA infection. Their related clinical features are summarized in table 2. These 42 patients comprised 25 males (59.5%) and 17 females (40.5%) with similar PVL\(^{+/-}\) rates (20% in males vs. 29.4% in females \([P = .7135, by Fisher’s exact test]\)). Moreover, the relative proportion of PVL\(^{+}\) and PVL\(^{-}\) isolates among these patients was not significantly different between the 4 different age groups \((P = .5541, by \chi^2 test)\), although the majority of these isolates (31/42 [73.8%]; 25.8% PVL\(^{+}\) vs. 74.2% PVL\(^{-}\)) were found in patients 18–65 years of age. Of the 42 patients, 12 (28.6%) had colonization only (1 PVL\(^{+}\) vs. 11 PVL\(^{-}\)), 18 (42.9%) had mild infections (4 PVL\(^{+}\) vs. 14 PVL\(^{-}\)), 10 (23.8%) had severe infections (4 PVL\(^{+}\) vs. 6 PVL\(^{-}\)), and 2 (4.8%) had unknown colonization/infection status (1 PVL\(^{+}\) vs. 1 PVL\(^{-}\)) (table 2). However, with regard to the propensity to cause minor infection or severe infection versus colonization, there was no significant difference between the PVL\(^{+}\) and PVL\(^{-}\) isolates \((P = .2082, by \chi^2 test)\).

PVL\(^{+}\) and PVL\(^{-}\) USA400 isolates possess similar virulence-gene profiles. To further characterize PVL\(^{+/-}\) USA400 strains, we performed PCR assays to determine the virulence-gene profiles for 11 toxin, 11 adhesin, and 3 exoenzyme genes in these isolates. Regardless of PFGE pattern, PVL\(^{+}\) and PVL\(^{-}\) strains possessed similar toxin, adhesin, and exoenzyme virulence-gene profiles, with all strains being observed (1) to carry the staphylococcal enterotoxin genes sea, sec, and sed but to lack the toxic shock–syndrome toxin gene tst and (2) to possess gamma-toxin gene hlg, fibronectin adhesive molecule gene fnbA but not fnbB, collagen adhesive–molecule gene cnaA, putative-adhesin gene sdrC/sdrD/sdrE, elastin-adhesin gene ebpS, major-histocompatibility-complex class II–analogue protein gene map/eap, clumping-factor gene clfA, polysaccharide intercellular-adhesin gene ica, coagulase gene coa, and serine-protease gene V8 (table 1).

Prophage \(\delta Sa2mw (the specific carrier of PVL genes) present in PVL\(^{+}\) but absent in PVL\(^{-}\) USA400 isolates. The PVL genes are carried in prophage \(\delta Sa2mw\) in the genome of the USA400 MW2 strain [31]. To understand the phenomena of
Table 2. Clinical features of hospital patients colonized/infected with Panton-Valentine leukocidin (PVL)⁺⁻ USA400 strains of community-associated (CA) and hospital-associated (HA) methicillin-resistant *Staphylococcus aureus* (MRSA).

<table>
<thead>
<tr>
<th>Strain (no. [%] of patients)</th>
<th>CA-/HA-MRSA</th>
<th>Sex</th>
<th>Age of patient, years</th>
<th>Clinical feature*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA-</td>
<td>HA-</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>PVL⁺ (10 [23.8])</td>
<td>7 (36.8)</td>
<td>3 (13.0)</td>
<td>5 (20.0)</td>
<td>5 (29.4)</td>
</tr>
<tr>
<td>PVL⁻ (32 [76.2])</td>
<td>12 (63.2)</td>
<td>20 (70.0)</td>
<td>20 (80.0)</td>
<td>12 (70.6)</td>
</tr>
<tr>
<td>Total (42 [100])</td>
<td>19 (45.2)</td>
<td>23 (54.8)</td>
<td>25 (59.5)</td>
<td>17 (40.5)</td>
</tr>
</tbody>
</table>

* For detailed classification scheme, see the Patients, Materials, and Methods section.
carriage of PVL genes and phage φSa2mw in the PVL+−
USA400 isolates, we conducted a comprehensive analysis and
alignment of the Staphylococcus-species genomes and pro-
phage sequences and determined that gene MW756 (coding
for a hypothetical protein) on genomic island ρSa3 of MW2 is
unique for the USA400 strain, whereas gene MW1409 (coding
for a hypothetical protein) is unique for prophage φSa2mw.
The specific primers for USA400 CA-MRSA strains and for
prophage φSa2mw in USA400 were designed and verified by
use of a comprehensive set of clinical isolates [28]. The sec-
ond pair of primers targeting gene MW1438, which is in an-
other region in prophage φSa2mw, was also designed. When
we used these primer sets, we determined that the USA400
strain-specific gene (i.e., MW756) was present in all 121
USA400 isolates. However, the prophage φSa2mw genes (i.e.,
MW1409 and MW1438) were present only in PVL+ isolates
and not in PVL− isolates, indicating that this prophage may
have been lost from these strains or may never have been
acquired by them (figure 3).

**DISCUSSION**

PVL is a bicomponent, pore-forming leukotoxin that, because of
its ability to lyse leukocytes, initially was designated “sub-
stance leukocidin” by Van deVelde in 1894. In 1932, Panton
and Valentine first associated the leukotoxin with skin and soft-
tissue infection, long before MRSA was of clinical concern [32].
PVL recently has been classified as belonging to the family of
synergohymenotropic toxins [33]. It causes leukocyte destruc-
tion and tissue necrosis and possesses chemotactic and proin-
flammatory properties [34, 35]. The PVL locus is carried on a
bacteriophage that is presently found in only a small proportion
(<5%) of S. aureus strains described worldwide [1, 34, 36].
Although the molecular basis for the enhanced virulence of
CA-MRSA compared with HA-MRSA is unknown, there is
strong association between CA-MRSA infections and the pres-
ence of PVL, suggesting that PVL may be a marker for CA-MRSA
[1, 37]. The presence of PVL in S. aureus appears to be associated
with increased disease severity, ranging from cutaneous infec-
tions requiring surgical drainage to severe chronic osteomyelitis,
deathly necrotizing pneumonia, and a recently described syn-
drome of severe sepsis with Waterhouse-Friderichsen syn-
drome [36, 38]. These observations, however, do not exclude the
possibility of coincidental coexistence of PVL genes in a virulent
genomic background of CA-MRSA strains. There is currently no
direct evidence to suggest that PVL plays a significant role in the
epidemiological features and pathogenesis of CA-MRSA infec-
tions.

Several studies have demonstrated that PVL could cause der-
monecrosis in animal models [39, 40]. Very recently,
Labandeira-Rey et al. [41] have reported that PVL alone is suf-
cient to cause necrotizing pneumonia in a mouse model of acute
pneumonia. However, Voyich et al. [42] have studied the PVL-
knockout strains of USA300 and USA400 in mouse models of
sepsis and abscess and have shown (1) that PVL− strains are as
lethal and virulent as wild-type strains and cause comparable
skin diseases and (2) that both lysis of human neutrophils and
pathogen survival after phagocytosis are similar in wild-type and
mutant strains. CA-MRSA strains in Western Australia, which
are predominantly MLST ST1 (i.e., the sequence type that cor-
responds to USA400) have historically been PVL−, with PVL
only recently having been detected in some isolates of this geno-
type [4, 43]. Moreover, in a population-based study, the fre-
cquent isolation of PVL− MRSA from the nares of asymptomatic
healthy carriers suggests that PVL is not required for dissemina-
tion via the respiratory route [44]. More recently, it has been
shown that PVL is not universally present in CA-MRSA strains
[45, 46].

Our previous work (based on testing of local clinical isolates
collected during the past 16 years) in the CHR [47] has demon-
strated that, in that geographic region, PVL+ isolates with di-
verse genomic backgrounds exist, including the currently de-
scribed CA-MRSA clones ST1-MRSA-IV (USA400 strain), ST8-
MRSA-IV (USA300 strain), ST59-MRSA-IV, ST80-MRSA-IV,
and ST30-MRSA-IV, as well as other MRSA strains (including
HA-MRSA) and methicillin-susceptible S. aureus, with variable
SCCmec types or subtypes (II and IVa–IVc). Interestingly, we
also found PVL genes in strains with genomic backgrounds sim-
ilar to those of typical epidemic HA-MRSA, strains that fre-
cquently were associated with SCCmec type II and MLST types
ST30, ST52, ST22 [47]. The results of the previous study pro-
vided indirect evidence that PVL genes are not necessarily the
key determinant(s) associated with newly emerging CA-MRSA
clones.

In the present study, we have reported the coexistence, within
a large health-care region, of PVL+− sibling USA400 strains
(with a predominance of the PVL− strain) that have identical
genotypic and phenotypic properties and similar clinical char-
acteristics. The results of this study provide direct evidence, from
a natural population of MRSA clinical isolates, that supports the
hypothesis that PVL genes are not necessarily the key determin-
ants associated with the increasing dissemination of CA-MRSA
strains, suggesting that the genomic milieu, particularly the rep-
ertoire of virulence and resistance genes, may play a greater role
in this regard.

Of the 121 inpatients from whom CA-MRSA USA400 strains
were isolated, there were only 42 who were hospitalized and for
whom medical records and detailed clinical information were
available. In these 42, the proportion of isolates that were PVL+
was 8% in colonized patients, 22% in patients with mild infec-
tion, and 40% in patients with severe infection (table 2). Al-
though these differences were not statistically significant, the
small number of patients for whom clinical information was
available limited the power to detect potentially important
severity-based differences in prevalence of PVL genes. Continued observation and case identification may allow us to detect additional PVL+/−-infected patients, allowing us to draw more solid conclusions regarding any potential association between PVL genes and severity of infection.

It has been well established that PVL genes (e.g., lukS- and lukF-PV) are encoded by a phage that integrates into the S. aureus chromosome. To date, 4 phages have been reported to carry PVL genes, including φSa2mw [48], φSLT [29], φSa2mw [31], and, most recently, φ108PVL [30]. The present study reveals that—except for prophage φSa2mw, which carries the PVL genes in USA400 strains but which is missing from PVL− USA400 isolates—PVL+/− strains share identical molecular characteristics and similar epidemiological and clinical features, suggesting that both PVL+ and PVL− USA400 strains are of the same clonal origin, with the only difference being the PVL gene-carrying prophage. However, we cannot determine which one of these is the ancestral strain. It is possible that the PVL− strain originated from the PVL+ strain via loss of prophage φSa2mw from the genome of the latter. Alternatively, the PVL+ strain may have...
descended from the PVL- strain by acquiring prophage \( \phi Sa2mw \). Further studies are necessary to reveal the biological and evolutionary relationships between and the significance of these 2 sibling strains.

### References


5. Centers for Disease Control and Prevention. Four pediatric deaths from *Staphylococcus aureus* strain by acquiring prophage \( \phi Sa2mw \). Further studies are necessary to reveal the biological and toxin genes in natural populations of *Staphylococcus aureus*. J Clin Microbiol 2005; 43:5026–33.


