Comparison of Virulence in Community-Associated Methicillin-Resistant Staphylococcus aureus Pulsotypes USA300 and USA400 in a Rat Model of Pneumonia

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Background. The predominant genetic background of community-associated methicillin-resistant Staphylococcus aureus has transitioned from USA400 to USA300 in most US communities. The explanation for this shift is unclear. We hypothesized that USA300 must be more pathogenic—specifically, that USA300 would have increased virulence when compared with USA400 in an animal model.

Methods. Rats were inoculated intratracheally with 1 of 6 S. aureus isolates from the USA300 and USA400 backgrounds. We assessed mortality, in vivo bacterial growth, and histopathology. We assessed the in vitro expression of capsule and of selected genes believed to be important in virulence in S. aureus, including agr, saeRS, sarA, α-toxin (hla), and Panton-Valentine leukocidin (pvl).

Results. USA300 isolates were more lethal, produced more severe pneumonia, and had higher in vivo bacterial density in the lung than did USA400 isolates. In vitro expression of agr, saeRS, sarA, hla, and pvl were greater in USA300 isolates. USA300 isolates were unencapsulated, whereas 2 of 3 USA400 isolates produced capsule.

Conclusions. USA300 isolates were more virulent than USA400 isolates in a model of necrotizing pneumonia. The explanation for this is unclear, but it likely results from increased expression of S. aureus regulatory systems (e.g., agr, saeRS, and sarA) and the resultant upregulation of key virulence factors including α-toxin and PVL.

Disease caused by community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) can be se-

vere, especially when it manifests as necrotizing pneumonia or severe sepsis [1–3]. When epidemic CA-MRSA disease was first described in several Midwestern medical centers, including Chicago [4], a genetic background now called USA400 [5] (as determined by pulsed-field gel electrophoresis [PFGE]) or multi locus sequence type (MLST) 1, was the major circulating clone [6]. A shift has occurred, however, and the current CA-MRSA background predominant in most US locales is USA300, or MLST 8 [7–9]. It is unclear why USA300 has become the predominant CA-MRSA background responsible for symptomatic infection in most of the United States.

Inspection of the published genome sequences of representative USA300 and USA400 strains [10–12] has revealed that methicillin resistance in both lineages is encoded by the mecA gene within the mobile genetic element staphylococcal cassette chromosome mec (SCCmec) type IV [13]. Also, these sequenced genomes contain the genes lukF-PV and lukS-PV, which encode the Panton-Valentine leukocidin (PVL) carried by nearly identical integrated prophages [10–12]. Based in part on this strong epidemiologic association, it has been
Some authors have postulated an important role for the S. cerevisiae rat model of pneumonia. Compared the virulence of these two backgrounds in a newly characterized rat model of pneumonia. Accordingly, we hypothesized that USA300 strains would be more virulent than USA400 strains in an animal model. Specifically, we expected that this epidemiologic observation might translate to dominance of USA300 strains in many communities, we expected virulence of USA300 and USA400 clinical isolates. Given the differential virulence in an animal model, postulated that PVL plays an important role in the pathogenesis of CA-MRSA pneumonia [14], but experimental infections in rodents have produced seemingly contradictory conclusions [15, 16]. Recently, α-hemolysin (hla) has been implicated as the major virulence factor in a murine model of CA-MRSA necrotizing pneumonia [17]. This is surprising because, although the sequenced genomes of both USA300 and USA400 lineages contain the hla gene, so do the majority of S. aureus clinical isolates [18]. Some authors have postulated an important role for the S. aureus polysaccharide capsule in invasive disease [19], but the role of capsule production in the pathogenesis of pneumonia is not known.

The sequenced genomes of USA300, but not those of USA400, contained an element called the arginine catabolic mobile element (ACME), which carries several genes thought to be associated with virulence, including those for oligopeptide permease-3 (opp-3) and arginine catabolism (arc) [10, 12]. The ACME element can also be found in the commensal coagulase-negative Staphylococcus epidermidis, an important component of human skin flora, but was not previously identified in S. aureus [10]. There are other differences between USA300 and USA400 isolates. For example, the collagen adhesin gene (cna) is present in USA400, but not in USA300 [10–12]. This is of interest because cna has been associated with increased binding of S. aureus isolates to basement membrane proteins, an event postulated to be important in the pathogenesis of necrotizing pneumonia [20].

There may also be differences in gene regulation. Burlak et al. noted that, compared with a USA400 isolate called MW2, a USA300 strain called LAC had increased abundance of several virulence factors, including hla, staphylokinase, enterotoxin K, and serine proteases [21].

To date, there has been no systematic study comparing the virulence of USA300 and USA400 clinical isolates. Given the dominance of USA300 strains in many communities, we expected that this epidemiologic observation might translate to differential virulence in an animal model. Specifically, we hypothesized that USA300 strains would be more virulent than USA400 strains in a rat model of pneumonia. Accordingly, we compared the virulence of these 2 backgrounds in a newly characterized rat model of S. aureus pneumonia with pathologic features identical to the fatal, necrotizing pneumonia that occurs in patients.

**METHODS.**

**Isolate selection.** Clinical isolates representing the MLST 1 lineage (presumed to represent USA400) and the MLST 8 lineage (presumed to represent USA300) were selected for evaluation (table 1) from the isolate library at the University of Chicago, which contains more than 1000 clinical S. aureus isolates categorized by clinical characteristics, antibiotic susceptibility data, and information from molecular typing, including evaluation for SCCmec type and MLST. The isolates selected were recovered from patients with a variety of CA-MRSA disease syndromes, including necrotizing pneumonia, severe sepsis, and abscesses. All isolates contained the lukS-PV and lukF-PV genes, and all USA300 isolates contained the arcA gene from ACME (see below). Strains MW2 (USA400) and LAC (USA300-0114) were kindly provided by Michael Otto (National Institutes of Health) [15].

**PFGE.** Whole cell DNA-containing agarose plugs were prepared and digested with Smal, as described elsewhere [5]. Restriction fragments were resolved on a CHEF DR-III PFGE system (Bio-Rad). A Smal digest of S. aureus strain 8325-4 (ATCC 35556) served as the molecular size standard. Strain LAC served as a standard for USA300, and MW2 served as a standard for USA400.

**Rat model of CA-MRSA pneumonia.** To prepare an animal inoculum, a frozen stock of S. aureus was subcultured onto tryptic soy agar (TSA) and incubated at 37°C overnight. A single colony was inoculated into 5 mL tryptic soy broth (TSB), shaken at 250 rpm, and incubated at 37°C overnight. The overnight culture was diluted 1:100 and grown to early stationary phase (OD₆₀₀ = 3.0). The cells were then centrifuged (3200 g for 10 min), washed in PBS, and resuspended in PBS. The growth curve of each isolate was similar (data not shown). All inocula were quantified by plating serial dilutions on TSA and enumerating colonies.

Animal experiments were conducted in accordance with the regulations of the University of Chicago Institutional Animal Care and Use Committee. A rat model of necrotizing pneu...
nia was established by modifying the method of Karzai et al. [22]. In brief, Sprague-Dawley rats (Harlan) weighing approximately 300 g were housed for 7 days prior to inoculation. Animals were anesthetized with ketamine and xylazine. The trachea was intubated with an 18-gauge angiocatheter (Becton-Dickinson) and anesthetized with pentobarbital at 42 h. Surviving animals were observed for signs of illness. All surviving animals were euthanized with pentobarbital at 42 h.

Animals were assigned to receive S. aureus at an inoculum of 1 × 10^4–2 × 10^6 cfu (“high inoculum”) or 4 × 10^2–5 × 10^5 cfu (“low inoculum”). Animals received 1 of 3 USA400 isolates (MW2, 649, or 2672) or 1 of 3 USA300 isolates (LAC, 923, or 2405) at high and low inocula (table 1).

**Quantitative culture and histopathology.** After sacrifice, the left lung was removed and placed in PBS. The lung was washed twice in PBS and homogenized (Tissuemiser; Fisher); serial dilutions of the homogenate were plated on mannitol salt agar for quantification of bacteria. The right lung was placed in PBS. The lung was homogenized (Tissuemiser; Fisher); serial dilutions of the homogenate were plated on mannitol salt agar for quantification of bacteria. The right lung was placed in PBS. The lung was homogenized (Tissuemiser; Fisher); serial dilutions of the homogenate were plated on mannitol salt agar for quantification of bacteria.

**Detection of toxin and virulence-factor genes in CA-MRSA isolates.** Isolates were analyzed for the presence of genes that encode several virulence factors thought to be important in experimental and clinical CA-MRSA pneumonia. The genes analyzed included arcA (representing ACME-arc) [23]; leukocidin DE; lukS-PV/lukF-PV; α-, β-, δ-, and γ-hemolysins [24]; and collagen adhesin (domains A and B) [25]. Bacterial isolates were subcultured onto TSA and grown overnight in TSB, as described above. Genomic DNA was isolated using the Qiagen DNAeasy kit modified by adding lysostaphin (100 μg/mL) to the resuspension buffer. Polymerase chain reaction (PCR) amplification was performed in a Bio-Rad Cycler (15 min at 95°C followed by 15 cycles of 30 s at 94°C, 90 s at 58°C, and 90 s at 72°C, and final extension for 10 min at 72°C). Agarose gel electrophoresis was performed to resolve the products. The presence or absence of a gene of interest was determined according to the molecular weight of the amplimer, compared with those obtained from control S. aureus strains.

**In vitro gene expression.** Frozen stock isolates were subcultured on TSA, incubated overnight in TSB, and diluted 1:100. Bacteria were harvested during logarithmic growth phase (3 h; OD₆₀₀ = 2.4) and early stationary growth phase (4 h; OD₆₀₀ = 3.5) and frozen in RNAProtect (Qiagen). Bacteria were lysed by use of the Fastprep FastRNA Pro Blue system (Q-BIOgene). Purification of RNA was achieved by using the Qiagen RNeasy kit, including treatment with DNase. cDNA was prepared by use of the high capacity cDNA Archive Kit (Applied Biosystems).

Northern blot analysis was performed to assess the expression of agr (RNAIII) [26], sarA [27], saeRS [28], and hla [24]. The specific probe was a gel-isolated PCR product labeled with [α-32P]dATP (Amersham) using the Prime-a-Gene labeling system (Promega). Densitometry was performed by using Molecular Imaging Software (version 4.0; Kodak). Net intensities were compared between pulsotypes.

Real-time reverse-transcriptase PCR (RT-PCR) was performed by use of primers and molecular beacons for lukF-PV and 16S rRNA as the endogenous control; the technique used was as described elsewhere [29], except that reactions were performed using an ABI Prism 7300 series RT-PCR thermocycler (Applied Biosystems). For hla, RT-PCR was performed by using light upon extension (LUX) with a forward hla-specific LUX primer labeled with 6-carboxy-fluorescin (FAM) (5’-CGGCACATTGGCACAATAGGC[FAMc]G-3’) (Invitrogen) and an unlabeled reverse primer (5’-GTTTTAGCCGTGCCCCTCAGC-3’). The LUX probe for the 16S rRNA endogenous control was labeled with 6-carboxy-4’,5’-dichloro-2’,7’-dimethoxy-fluorescin (JOE) (5’-CGGCCTAATCGTGCCAGCAGC[JOEc]G-3’), and the reverse primer was unlabeled (5’-GCCGTTCAGGCCCCAATATTCC-3’). Primers were validated by using standard curves generated for hla and the 16S rRNA with known quantities of S. aureus genomic DNA (data not shown). Cycling conditions were identical to those used for lukF-PV [29]. Data were analyzed by use of the ABI Prism 7300 Sequence Detection Software (version 1.2.3; Applied Biosystems).

### Table 2. Pathologic scoring system to grade severity of pneumonia.

<table>
<thead>
<tr>
<th>Criterion, description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Largest focus of inflammation</strong></td>
<td></td>
</tr>
<tr>
<td>Small (&lt;1 low power field)</td>
<td>1</td>
</tr>
<tr>
<td>Medium (1-2 but &lt;1 low power field)</td>
<td>2</td>
</tr>
<tr>
<td>Large (1 low power field)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Distribution and extent of lesions</strong></td>
<td></td>
</tr>
<tr>
<td>Mild (&lt;10% of sample)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate (10%-50% of sample)</td>
<td>2</td>
</tr>
<tr>
<td>Severe (&gt;50% of sample)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Necrosis</strong></td>
<td></td>
</tr>
<tr>
<td>None/mild (&lt;10% of sample)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate (10%-50% of sample)</td>
<td>2</td>
</tr>
<tr>
<td>Severe (&gt;50% of sample)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Colonies</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Single</td>
<td>1</td>
</tr>
<tr>
<td>Multifocal</td>
<td>2</td>
</tr>
<tr>
<td><strong>Classification by total score</strong></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>0–4</td>
</tr>
<tr>
<td>Moderate</td>
<td>5–8</td>
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<tr>
<td>Severe</td>
<td>9–11</td>
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</tbody>
</table>

**Note.** Samples were scored by a blinded observer trained in pathology.
Biosystems). Quantification was performed relative to the 16S rRNA probe.

**Capsule phenotype and genotype.** The capsules of the *Staphylococcus aureus* strains were typed by colony immunoblotting [30]. The results were confirmed by evaluating the reactivity of capsule extracts prepared from each strain by double immunodiffusion against polyclonal antibodies specific for CP5 or CP8, as described elsewhere [31]. Amplification and sequencing of specific *cap5* (8) gene regions was performed as described elsewhere [32].

**Statistical analysis.** Mortality data were analyzed by using Fisher's exact test. Weight loss, quantitative culture, and expression data were compared by Student's *t* test. Pathology scores were compared by the Mann-Whitney *U* test (SPSS-W, version 15; SPSS). *P* < .05 was considered to be statistically significant.

**RESULTS**

**PFGE.** The MLST and pulsotype of the strains correlated as expected. All MLST 1 strains were identified as USA400 by PFGE (figure 1). Similarly, all MLST 8 strains were identified as USA300.

**Rat model.** Four groups of animals (18–23 per group for each mortality study, and 12–15 per group for the other studies) were inoculated with USA300 and USA400 isolates at high and low inocula, respectively. Eight control animals received PBS. All inoculated animals, but no control animals, appeared ill within 3 h, with rapid, labored breathing. Because preliminary data demonstrated that weight loss among surviving rats reached a nadir at 42 h, after which surviving animals recover (data not shown), all surviving animals were sacrificed at that time. Among the low inoculum recipients, animals infected with USA300 strains lost more weight than did those inoculated with USA400 isolates; the USA300 strain–infected animals lost a mean of 12% of their original body weight, whereas those infected with USA400 isolates lost a mean of 8% of their original body weight (*P* < .05) (figure 2A).

**Mortality.** Infection with the high inoculum of the USA300 strains was uniformly lethal; the mortality rate was lower among animals that received the high inoculum of the USA400 strains (37%) (*P* < .01) (figure 3A). Mortality was also more frequent among animals that received the low inoculum of USA300 strains, compared with those that received the low inoculum of USA400 strains (38% vs. 0%; *P* = .02) (figure 3B). Death occurred quickly in all animals who succumbed, typically within 3–12 h of inoculation, and always within 24 h of inoculation. The LD₅₀ values were 7 × 10⁶ cfu and >2 × 10⁹ cfu for the USA300 and USA400 isolates, respectively (data not shown). No control animals died.

**Tissue pathology.** The lungs of all animals inoculated with *S. aureus* were heavier than those of control animals. Most lungs from animals infected with USA400 strains were dark pink and mottled (figure 4B), without necrosis. The lungs of animals infected with USA300 strains (and several animals infected with USA400 isolates at high inocula) that survived to 42 h had necrotic areas that overlaid hemorrhagic appearing lung (figure 4C). Animals who died had congested lungs (figure 4D).

Compared with lungs from control animals (figure 4E and 4F), histologic examination of lungs from animals infected with USA400 strains typically revealed consolidated airspaces with an inflammatory infiltrate, but few bacterial colonies and no necrosis (figure 4G and 4H). Pathology scores were consistently higher for

![Figure 1.](https://academic.oup.com/jid/article-abstract/198/4/561/832926)
the lungs of animals infected with USA300 strains (P < .001) (figure 2B), reflecting more extensive lesions, as well as the more frequent presence of bacterial colonies and necrosis (figure 4I and 4J). Lungs from several animals infected with USA400 isolates (high inoculum only) also showed severe, necrotizing pneumonia similar in appearance to that seen in the lungs of animals infected with USA300 strains, but this occurred significantly less often in animals infected with USA400 (P < .01) (figure 2B).

**In vivo bacterial growth.** Animals infected with USA300 isolates at the high inoculum had a mean 10-fold higher bacterial density in the lungs than did those similarly inoculated with USA400 (P < .01) (figure 2C). When infected with USA300 isolates at the high inoculum, the typical lung quantitative culture yielded >2 × 10^9 cfu/lung. Assuming bacterial density was similar in the 2 lungs of any given animal, in vivo bacterial recovery was greater than the amount inoculated, indicating that in vivo replication occurred. Among animals that received the low...
ocula, bacterial counts were >100-fold higher among those infected with USA300 strains, compared with animals infected with USA400 strains \( (P = .03) \) (figure 2C). Cultures of samples from the lungs of control animals who received PBS yielded no bacterial growth.

**Virulence factor detection.** Irrespective of pulsotype, all isolates contained the genes *hla, hlb, hld, hlg, lukED,* and *lukSF-PV* (data not shown). USA400 isolates, but not USA300 isolates, contained *cna,* whereas USA300 isolates, but not USA400 isolates, contained ACME-arcA.

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**Figure 4.** Gross pathology of lungs from normal and *Staphylococcus aureus*–infected animals (A–D) and hematoxylin-eosin stained sections of lung tissue (E–J). A, Normal lung from an animal inoculated with PBS. B, Consolidated lung from an animal infected with a representative USA400 isolate. C and D are lungs from animals inoculated with a representative USA300 isolate. C, Overt necrosis (arrow) with underlying hemorrhage. D, Dense, congested lung from an animal that died. E and F, Sections of normal lungs from animals inoculated with PBS; G and H, Alveolar consolidation and neutrophilic infiltration of airspaces in the lungs of animals infected with USA400 strains. I and J, Infection with USA300 strains, showing typical necrotizing pneumonia with multifocal bacterial colonies, complete destruction of alveolar architecture, hemorrhage, and perivascular growth of *S. aureus* (arrow). Several animals infected with USA400 strains at the high inoculum showed similar necrosis. Panels E, G, and I are 4× magnification; panels F, H, and J are 40×.
USA300 strains carry the \textit{cap5} locus [10, 12], but none of the USA300 isolates that we used produced CP5. Therefore, we amplified and sequenced a 600-bp region of the \textit{cap5} locus that includes the \textit{cap5A} promoter region in all USA300 isolates. All amplicons carried a single nucleotide mutation (T to C) within the conserved inverted repeat upstream of \textit{cap5A}, which abrogates \textit{cap5} promoter activity [33]. We also amplified and sequenced a 854-bp region of \textit{cap5D} from all USA300 isolates. Each strain had the identical frameshift mutation documented in the sequenced USA300 strains [10, 12], which results in premature termination during translation of the full-length protein. The \textit{cap5A} promoter and \textit{cap5D} mutations had been characterized previously from \textit{S. aureus} clinical isolates and resulted in loss of capsule production [32].

USA400 strains carry the \textit{cap8} locus [11], but we only detected capsule production by strains MW2 and 2672. Most clinical isolates that fail to produce capsule carry point mutations within the \textit{cap5(8)} locus [32]. Strains MW2 and 649 carry a frameshift mutation in \textit{cap8A} that results in a truncated version (171 aa) of the full-length gene product (222 aa). \textit{Cap5A} is thought to regulate polymer chain length [34]; this belief is supported by the fact that the capsule produced by strain MW2 was reduced in size, compared with that of other MLST 1 isolates (data not shown).

Transcription. By Northern blot analysis, expression of \textit{agr} (RNAIII) ($P \leq 0.05$) and \textit{saeRS} ($P \leq 0.02$) was shown to be greater among USA300 isolates than among USA400 isolates, at both 3 h and 4 h (figure 5C). Compared with the USA400 iso-
lates, there was also greater sarA expression at 3 h among the USA300 isolates (P < .02) (figure 5C), but not at 4 h (P = .3). USA300 isolates expressed lukF-PV at higher levels than did 2 of the 3 USA400 isolates, but the difference was not significant (P = .12) (figure 5A). Isolate 2672, a methicillin-susceptible isolate, was an outlier among the USA400 isolates; it expressed lukF-PV at levels similar to those of the USA300 isolates. RT-PCR showed that USA300 isolates expressed hla at much higher levels than did the USA400 isolates (P < .001) (figure 5B). This difference was validated by Northern blot analysis; in fact, hla expression was nearly undetectable among USA400 isolates (figure 5C) (P < .01).

**DISCUSSION**

USA300 isolates were more virulent than USA400 strains in this model, as evidenced by increased lethality, greater intrapulmonary bacterial growth, and more severe pathology. Bubeck-Wardenburg et al. also suggested that LAC was more lethal than MW2 in a murine model of pneumonia [17], although they were not directly compared. These findings contrast with those of Voyich et al., who found that USA300 and USA400 isolates produced similar mortality in mice after intravenous inoculation [15]. Those investigators also found that intradermal injection of strain MW2 produced well-formed abscesses, but similar injection of strain LAC produced dermonecrosis. These differences suggest that the route of inoculation influences the pathogenesis of *S. aureus* infection.

Intratracheal inoculation of rats with CA-MRSA isolates caused illness with reproducible clinical and pathologic features. Importantly, histopathologic analysis of lung tissue revealed necrotizing pneumonia resembling that documented in postmortem tissue from patients [2] and was characterized by obliteration of alveolar architecture, perivascular accumulation of *S. aureus*, and hemorrhage. The presence of pulmonary hemorrhage is noteworthy, because hemorrhage has recently been identified as an important risk factor in predicting mortality in patients with CA-MRSA necrotizing pneumonia [35].

The mechanisms underlying the increased virulence of USA300 isolates, compared with that of USA400 isolates, are uncertain. The presence or absence of an essential virulence factor was one possibility we considered. For example, the cna gene was present only in the less-virulent USA400 isolates, suggesting that cna does not augment virulence in this pneumonia model. The arcA gene encoded in ACME was unique to the USA300 strains in this study. The virulence contribution of this element has not yet been defined, but it has been hypothesized to play a role [10]. Although capsule production has been reported to enhance *S. aureus* virulence, the strains in this study (except for MW2 and 2672) did not produce detectable capsule.

We found differences in transcriptional regulation that might explain, in part, the differences in virulence that we observed. The USA300 isolates expressed the global regulators agr (RNAIII), saeRS, and sarA at higher levels than did the USA400 isolates. These global regulators influence the expression of many exoprotein genes, including toxins and adhesins associated with virulence [36–38]. SarA, saeRS, and agr have been shown to influence the expression of hla [39]. Upregulation of global regulators might also explain the findings of Burlak et al [20], who found increased abundance of multiple virulence factors, including α-toxin, in a USA300 background.

Not surprisingly, we also found that USA300 isolates uniformly expressed hla at higher levels than did USA400 isolates. Several studies have documented an important role for α-toxin in rodent models of pneumonia [17, 40]. A role for α-toxin in virulence has been proposed for many years, but few data exist that suggest relative transcription might increase virulence in experimental infection. Koo et al. reported that hyperproduction of α-toxin by *S. aureus* actually decreased virulence in a rabbit model of catheter-induced endocarditis [41], presumably due to the release of platelet microbicidal proteins. The increased virulence seen with USA300 isolates in the rat model was associated with increased hla transcription. At the high inoculum level, USA400 isolates only occasionally caused necrotizing pneumonia in our model (and in patients) despite relatively low in vitro hla expression.

Pvl expression was also higher in USA300 isolates, compared with expression in 2 of the 3 USA400 isolates. There has been a strong epidemiologic association between PVL and invasive MRSA disease [3, 14], but demonstrating a role for PVL in virulence has proved difficult. Voyich et al. and Bubeck-Wardenburg et al. found no difference in wild-type strains, compared with PVL-deletion mutants, in murine models of pneumonia, bacteremia, and skin abscesses [15, 17]. However, Labandeira-Rey et al. demonstrated that inserting the pvl genes into a less-virulent *S. aureus* isolate (RN4220) increased virulence and produced pneumonia. Moreover, direct instillation of PVL protein produced necrotizing pneumonia in our model (and in patients) despite relatively low in vitro hla expression.

It should be noted that isolate 2672, the sole methicillin-susceptible isolate used in this study, was an outlier. Although PFGE shows it to be USA400, it expressed lukF-PV and saeRS at higher levels than did the methicillin-resistant USA400 isolates. Interestingly, this did not translate into increased virulence.

Our study has several limitations. It is likely that there are important species-specific differences in the host response to
experimental S. aureus infection. Also, the intratracheal route of inoculation, bypassing the nasopharynx, may not be medically relevant. Additionally, in vitro expression of S. aureus virulence factors may not correlate with the expression that occurs in vivo. The USA300 and USA400 backgrounds also likely differ with respect to other uncharacterized virulence factors. For example, Wang et al. recently identified peptides called “phenol-soluble modulins” (PSMs) as important determinants of CA-MRSA’s virulence in bacteremia and skin abscesses [42]. They did not, however, demonstrate differences in PSM production between the USA400 and USA300 isolates they studied. Finally, although it is clear that USA300 has replaced USA400 as the predominant pulsotype in many communities, epidemicity (i.e., the survival and spread of a bacterial strain in the community) may not equate with virulence. Whether the same factors that caused increased virulence in the rat model are also responsible for success in the community remains unclear.

In summary, USA300 isolates were more virulent than USA400 isolates in a rat model of S. aureus necrotizing pneumonia that bears a striking similarity to clinical disease seen in humans. These isolates expressed several global regulators and virulence genes at high levels in vitro, which likely explains, at least in part, their increased virulence.

Acknowledgments

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