Illness Severity in Community-Onset Invasive Staphylococcus aureus Infection and the Presence of Virulence Genes


Background. It is uncertain whether particular clones causing invasive community-onset methicillin-resistant and methicillin-sensitive Staphylococcus aureus (cMRSA/cMSSA) infection differ in virulence.

Methods. Invasive cMRSA and cMSSA cases were prospectively identified. Principal component analysis was used to derive an illness severity score (ISS) from clinical data, including 30-day mortality, requirement for intensive hospital support, the presence of bloodstream infection, and hospital length of stay. The mean ISS for each S. aureus clone (based on MLST) was compared with its DNA microarray-based genotype.

Results. Fifty-seven cMRSA and 50 cMSSA infections were analyzed. Ten clones caused 82 (77%) of these infections and had an ISS calculated. The enterotoxin gene cluster (egc) and the collagen adhesin (cna) gene were found in 4 of the 5 highest-ranked clones (ST47-MSSA, ST30-MRSA-IV[2B], ST45-MSSA, and ST22-MRSA-IV[2B]) compared with none and 1 of the lowest 5 ranked clones, respectively. cMSSA clones caused more severe infection than cMRSA clones. The lukF/lukS Panton–Valentine leukocidin (PVL) genes did not directly correlate with the ISS, being present in the second, fourth, and 10th most virulent clones.

Conclusions. The clinical severity of invasive cMRSA and cMSSA infection is likely to be attributable to the isolates’ entire genotype rather than a single putative virulence determinant such as PVL.

Over the last decade, increasing rates of invasive community-onset methicillin resistant Staphylococcus aureus (cMRSA) infection have been reported worldwide [1–4]. Panton–Valentine leukocidin (PVL) is considered an important virulence factor in S. aureus, particularly in cMRSA pneumonia [5, 6], and is present in the most common cMRSA clones in the United States (ST8-MRSA-IV[2B]/USA300) [7] and continental Europe (ST80-MRSA-IV[2B]) [8]. Other virulence factors in S. aureus include the superantigen (SAg) family (eg, toxic shock syndrome toxin-1 and the staphylococcal enterotoxins), and adhesion proteins like collagen adhesin protein (cna). Severe infection caused by S. aureus strains containing these genes have been previously reported [9–12].

Relatively few studies have directly compared invasive cMRSA to invasive community-onset methicillin susceptible Staphylococcus aureus (cMSSA) infection [13–15]; consequently, it remains unclear whether MRSA is inherently more virulent than MSSA [16]. Recently, we found no difference in 30-day mortality between patients with invasive PVL-positive and -negative cMRSA and cMSSA infection, and noted severe cases of both invasive cMRSA and cMSSA infection caused by isolates lacking the genes for PVL and occurring in patients without obvious risk factors for poor outcome [17].
We hypothesized that virulence factors other than PVL may be important in determining the severity of an invasive infection due to cMRSA or cMSSA. The aim of this study was to examine markers of illness severity associated with invasive community-onset S. aureus infection, devise a simple illness severity score (ISS) that could be used to compare frequently isolated S. aureus clones in terms of illness-causing severity, and compare the mean ISS for each clone with the presence of genes encoding a wide range of known virulence determinants.

METHODS

Patients and Study Design
Fifty-seven patients who required hospital admission with invasive cMRSA infection were prospectively identified during a 2-year period at 2 teaching hospitals in Western Australia as described previously [17]. For each cMRSA case, 2 contemporaneous invasive cMSSA cases were selected. The study was approved by the Ethics Committees of both institutions.

Definitions
Invasive infection was defined as infection of a sterile site, or a skin and soft tissue infection (SSTI) requiring hospital admission for surgical drainage/debridement under general anaesthetic and/or intravenous antimicrobial therapy. “Community-onset” infection was defined as infection present or incubating on admission or detected within 48 hours of admission and not a complication of an indwelling device, surgical procedure, invasive instrumentation, or chemotherapy-related neutropenia [18].

Clinical Data Collection
Clinical data were obtained from medical records and laboratory and clinical information systems. Demographic information included age, gender, and Aboriginal ethnicity (as identified by the patient). Potential risk factors for invasive staphylococcal infection, including diabetes mellitus, intravenous drug use (IVDU), history of Staphylococcus aureus infection, hospitalization in the last 12 months, and residence in a long-term care facility (LTCF) were recorded [19, 20]. Presenting symptoms recorded included fever, rigors, back pain, arthralgias and/or myalgias, and confusion. Clinical syndromes at the time of presentation were categorized as (1) SSTI; (2) musculoskeletal infection (MSI), which included osteomyelitis, septic arthritis, bursitis, tenosynovitis, and epidural abscess; (3) community-acquired pneumonia (CAP), which required radiographic changes in keeping with pneumonia and either positive respiratory cultures (spumum, pleural fluid, or bronchoalveolar lavage) or bloodstream infection (BSI) in the absence of another focus of infection; (4) infective endocarditis (IE) defined as a case satisfying modified Duke criteria for “definite IE” [21]; and (5) primary (source unclear) BSI and secondary (probable source present) BSI.

In addition to BSI, markers of illness severity that were recorded included clinical and laboratory features to calculate the Simplified Acute Physiology Score II (SAPS II) [22], requirement for intensive care unit or high-dependency area (ICU/HDA) admission, length of hospitalization, and 30-day all-cause mortality.

Laboratory Data Collection
Confirmation of MRSA identification was performed by detection of mecA and nuc genes by multiplex polymerase chain reaction (PCR) [23]. Contour-clamped homogeneous electric field (CHEF) electrophoresis was performed on all cMRSA isolates and on the first 50 cMSSA isolates using the CHEF DR III System (Bio-Rad Laboratories Pty Ltd) [24]. Multilocus sequence typing (MLST) was performed on representative isolates with novel CHEF pulsortypes [25]. To assign a sequence type, the sequences obtained were compared with the sequences described on the MLST website (http://saureus.mlst.net/). Isolates were tested for the presence of the PVL lukS-PV and lukF-PV genes by PCR [26]. Staphylococcal cassette chromosome mec (SCCmec) allotyping was performed using previously published primers that identified the class of mec complex and type of cassette chromosome recombinase (ccr) complex encoded on the element [27]. Structural architecture of the SCCmec was determined with multiplex PCR primers [28]. Staphylococcus aureus nomenclature was based on sequence type (ST) generated by the MLST database and, for MRSA, the SCCmec type. Finally, DNA microarray (PM7 version, Alere, Jena, Germany) was performed on all isolates belonging to the 10 most common clones causing invasive MRSA and MSSA infection and detected the presence or absence of 334 target sequences (185 distinct genes and their allelic variants encoding known virulence and adhesion factors) [29].

Statistical Analysis
Principal components analysis (PCA) with varimax rotation [30] was performed on 4 variables from the patient data set: 30-day all-cause mortality (died = 1, alive = 0), requirement for intensive care unit/high-dependency area (ICU/HDA) support (yes = 1, no = 0), BSI rate (yes = 1, no = 0), and hospital length of stay (LOS) in days. As LOS was skewed, this variable was natural log-transformed prior to analysis. The PCA produced 2 components, each with a distinct set of regression weights measuring the relative importance of the original variables to the component. These weights were used to calculate a score for each patient on each component. For each set of component scores, the mean was zero and the standard deviation was 1. Scores on the first component were used as measures of
illness severity, with scores above zero indicative of more severe illness.

A mean illness severity score (ISS) was calculated for the 10 most common S. aureus clones (causing infection in at least 4 patients) and compared with patient demographics, risk factors, clinical syndrome, and virulence profile. These 10 common clones were further divided into the 5 highest- and 5 lowest-ranking clones according to their mean ISS and compared on dichotomous host characteristics using Fisher’s exact test and on continuous host characteristics using the Wilcoxon rank sum test.

PCA was performed using SPSS version 16.0 (SPSS, Chicago, IL); all other analyses were performed using Stata version 10.0 (Stata Corporation, College Station, TX).

RESULTS

CHEF and MLST Typing and Clinical Correlation of Isolates

CHEF and MLST types were assigned to all 57 cMRSA isolates and the first 50 cMSSA isolates (Figure 1 and 2). Eight clones caused invasive cMRSA infection, in contrast to 18 for invasive cMSSA infection. The 10 most common clones causing invasive S. aureus infection accounted for 82/107 (77%) of infections (54 cMRSA [6 clones] and 28 cMSSA [4 clones]).

Patient demographics and risk factors for the 10 most prevalent S. aureus clones are reported in Table 1. The median age of patients ranged from 23 years for ST93-MRSA-IV[2B] infections, to 80 years for ST22-MRSA-IV[2B] (EMRSA-15) infections. A limited number of clones caused invasive infection in Aboriginal patients (5), those from LTCFs (5), or in association with IVDU (6).

Clinical syndromes for the 10 most common S. aureus clones are presented in Table 2. BSI occurred predominantly in 3 clones: ST45-MSSA (71%) and ST47-MSSA (80%), both belonging to clonal complex (CC) 45; and ST22-MRSA-IV[2B] (80%). Three of the 4 cases of IE were caused by CC45-MSSA clones. ST93-MRSA-IV[2B] was the only clone that did not cause BSI, causing only SSTI and MSI.

Illness Severity Scores (ISS) of S. aureus Clones

PCA produced 2 components accounting for 75% of the variance in the 4 variables that were analysed (the SAPS II score did not add to the explained variance and so was excluded). BSI, ICU/HDA, and 30-day mortality had high loadings on the first component (all $r > 0.66$), while hospital length of stay (LOS) had a high loading on the second component ($r = 0.93$). The first component was used as an indicator of severity of infection and the component score coefficients were used to calculate an ISS for each patient. The equation for calculating ISS was:

$$\text{ISS} = 0.50 \times \text{30-day mortality} + 0.47 \times \text{HDA/ICU} + 0.38 \times \text{BSI} - 0.03 \times \ln \text{LOS}.$$

If a patient was alive after 30 days, was not admitted to HDA/ICU, did not have a BSI, and was hospitalized for 10 days (natural log = 2.3), then the patient’s ISS would be $-0.069 (0.50 \times 0 + 0.47 \times 0 + 0.38 \times 0 - 0.03 \times 2.3)$. As this value is less than zero, the patient would be at the lower end of the severity scale.

The mean ISS was calculated for each of the 10 most common clones and summary statistics for the individual variables that comprise the PCA in addition to the mean SAPS II scores for each clone are included in Table 3. ST47-MSSA (PVL negative), ST30-MRSA-IV[2B] (PVL positive), and ST45-MSSA (PVL negative) had the 3 highest ISS values (1.114, 0.481, and 0.394), while ST93-MRSA-IV[2B] (PVL positive) had the lowest ($-0.655$). When the 107 S. aureus
clones were categorized by MRSA/MSSA status, the mean ISS was higher for MSSA isolates compared with MRSA isolates (0.238 vs −0.209; \( P = .001 \)).

**Microarray-Based Genotype**

DNA microarray was performed on all 82 isolates belonging to the 10 most common MSSA and MRSA clones and on representative strains of the less common MSSA and MRSA clones. The higher ranking clones belonged to \( agr \) type I and III. Capsule type 8 was seen in 8/10 clones (Table 4).

**Adhesion Factors**

All isolates possessed numerous adhesion factors with \( clfA, clfB, fib, \) and \( fnbA \) universally detected in the 10 most common clones (Table 4). The adhesion factor \( fnbB \) was not detected in ST22-MRSA-IV[2B]; \( cna \) was only detected in 4 of the 5 highest ranked clones (the exception being ST93-MSSA).

**Haemolysins and Leukocidins Including PVL**

The majority of the most common clones had several haemolysins (Table 4). Haemolysin \( \alpha \) and \( \delta \) genes were universally detected. The haemolysin \( \beta \) gene was detected in most of the common clones except ST45-MSSA and ST5-MRSA-IV[2B], while the genes for haemolysin \( \gamma \) were found in all isolates except ST93-MRSA-IV[2B] and ST93-MSSA. The \( lukF/lukS \) PVL genes were detected in the minority of MRSA (3/8) and MSSA (2/18) clones comprising 32% and 22% of all MRSA and MSSA isolates, respectively (Figure 1 and 2 and Table 4). Among the ranked clones, PVL genes were detected in ST30-MRSA-IV[2B] (rank 2), ST93-MSSA (rank 4) and ST93-MRSA-IV[2B] (rank 10). Leukocidins D and E were only detected in a number of the lower-ranked clones.

**Enterotoxins**

Enterotoxin genes were also detected in the majority of the most common \( S. aureus \) clones and were more common in those with a higher ISS (Table 4). Four of the 5 top ISS ranking clones (ST47-MSSA, ST30-MRSA-IV[2B], ST45-MSSA, and ST22-MRSA-IV[2B]) contained the \( egc \) in contrast to 1 of the 5 lowest-ranking clones. In addition to the ranked clones, the \( egc \) was detected in a number of the less common unranked clones: ST45-MRSA-IV[2B], ST5-MSSA(CC5), ST34-MSSA(CC30), ST72-MSSA(CC72), ST73-MSSA(CC5), ST121-MSSA(CC121), and ST920-MSSA(CC5). Two deaths were observed among those with less common clones, both of which contained the \( egc \) alone (ST73-MSSA) or in combination with \( sea \) (ST5-MSSA), and both of which were PVL negative. The \( egc \) was more common among MSSA isolates (45%) compared with MRSA isolates (17%).

**Table 1. Summary of Demographic Data and Risk Factors for 10 Most Prevalent \( S. aureus \) Clones**

<table>
<thead>
<tr>
<th>Clone</th>
<th>Clonal Complex (CC)</th>
<th>n</th>
<th>Median Age, Years</th>
<th>Male Gender</th>
<th>Aboriginal</th>
<th>DM</th>
<th>IVDU</th>
<th>Previous ( S. aureus ) Infection</th>
<th>Recent Hospitalization</th>
<th>LTCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST1-MRSA-IV[2B]</td>
<td>1</td>
<td>15</td>
<td>48 (40)</td>
<td>6 (40)</td>
<td>4 (27)</td>
<td>6 (40)</td>
<td>5 (33)</td>
<td>5 (33)</td>
<td>9 (60)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>ST93-MRSA-IV[2B]</td>
<td>Singleton</td>
<td>13</td>
<td>23 (77)</td>
<td>7 (54)</td>
<td>1 (8)</td>
<td>2 (15)</td>
<td>3 (23)</td>
<td>5 (38)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ST78-MRSA-IV[2B]</td>
<td>88</td>
<td>12</td>
<td>63 (60)</td>
<td>6 (60)</td>
<td>2 (17)</td>
<td>4 (33)</td>
<td>3 (25)</td>
<td>5 (42)</td>
<td>2 (17)</td>
<td></td>
</tr>
<tr>
<td>ST93-MSSA</td>
<td>Singleton</td>
<td>10</td>
<td>43.5 (80)</td>
<td>8 (80)</td>
<td>4 (40)</td>
<td>2 (20)</td>
<td>2 (20)</td>
<td>1 (10)</td>
<td>1 (10)</td>
<td>0</td>
</tr>
<tr>
<td>ST45-MSSA</td>
<td>45</td>
<td>7</td>
<td>55 (71)</td>
<td>5 (71)</td>
<td>3 (43)</td>
<td>2 (29)</td>
<td>1 (14)</td>
<td>1 (14)</td>
<td>1 (14)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>ST15-MSSA</td>
<td>15</td>
<td>6</td>
<td>65 (83)</td>
<td>5 (83)</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>0</td>
<td>1 (17)</td>
<td>2 (33)</td>
<td>0</td>
</tr>
<tr>
<td>ST22-MRSA-IV[2B]</td>
<td>22</td>
<td>5</td>
<td>80 (80)</td>
<td>4 (80)</td>
<td>0</td>
<td>1 (20)</td>
<td>0</td>
<td>1 (20)</td>
<td>4 (80)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>ST5-MRSA-IV[2B]</td>
<td>5</td>
<td>5</td>
<td>68 (60)</td>
<td>3 (60)</td>
<td>3 (60)</td>
<td>4 (80)</td>
<td>0</td>
<td>2 (40)</td>
<td>3 (60)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>ST47-MSSA</td>
<td>45</td>
<td>5</td>
<td>57 (80)</td>
<td>4 (80)</td>
<td>1 (20)</td>
<td>0</td>
<td>0</td>
<td>1 (20)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ST30-MRSA-IV[2B]</td>
<td>30</td>
<td>4</td>
<td>43 (25)</td>
<td>1 (25)</td>
<td>0</td>
<td>0</td>
<td>2 (60)</td>
<td>1 (25)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are number (%) unless otherwise specified.
Abbreviations: DM, diabetes mellitus; IVDU, intravenous drug use; LTCF, long-term care facility.
Other recognized combinations of enterotoxins (sed + sej + ser, sec + sel, and sek + seq) were detected in a number of clones. The genes for sed + sej + ser (in addition to egc and sec + sel) were detected only in one ST47-MSSA isolate from a 60-year-old female with extensive epidural abscess and rapid death (after 2 days). Apart from ST45-MSSA and ST47-MSSA isolates, the sec + sel combination was also detected in ST22-MRSA-IV[2B] (ISS rank 5) and ST78-MRSA-IV[2B] (ISS rank 6) and the uncommon ST12-MSSA(CC12) and ST78-MRSA (CC88) clones. The combination of sek + seq was detected in ST1-MRSA-IV[2B] (rank 7) and the uncommon ST45-MRSA-IV[2B], ST8-MRSA-IV[2B] (USA300), and ST59-MSSA(CC59) isolates.

In addition to being present in ST1-MRSA-IV[2B] (rank 7) and ST5-MRSA-IV[2B] (rank 9), sea was also detected in ST5-MSSA, ST8-MSSA, ST12-MSSA, and ST920-MSSA. The enterotoxin seb was detected in a minority of ST45-MSSA (rank 3), ST22-MRSA-IV[2B] (rank 5), and ST78-MRSA-IV[2B] (rank 6) isolates. The enterotoxin seh occurred in ST1-MRSA-IV[2B] (rank 7) and ST34-MSSA. Enterotoxin see was not detected. Toxic shock syndrome toxin-1 (tst1) was detected in one ST45-MSSA isolate and some less common clones (ST45-MRSA-IV[2B], ST34-MSSA, ST121-MSSA, and ST920-MSSA). Only ST5-MRSA-IV[2B] was positive for the epidermal differentiation inhibitor gene edinA. No genes for epidermolytic toxins A, B, and D were detected. Arginine catabolic mobile element (ACME) was detected in 1 ST22-MRSA-IV[2B] isolate.

### Host Factors Categorized by High and Low ISS-Ranking Clone

There was little statistical difference in host factors between those patients having a high versus low ISS-ranking *S. aureus* clones.

### Table 2. Clinical Syndromes of 10 Most Prevalent *S. aureus* Clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>n</th>
<th>SSTI (%)</th>
<th>MSI (%)</th>
<th>CAP (%)</th>
<th>IE (%)</th>
<th>Primary BSI (%)</th>
<th>Secondary BSI (%)</th>
<th>Primary + Secondary BSI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST1-MRSA-IV[2B]</td>
<td>15</td>
<td>3 (20)</td>
<td>8 (53)</td>
<td>2 (13)</td>
<td>1 (7)</td>
<td>1 (7)</td>
<td>3 (1 SSTI, 1 MSI, 1 IE)</td>
<td>4 (27)</td>
</tr>
<tr>
<td>ST93-MRSA-IV[2B]</td>
<td>13</td>
<td>9 (69)</td>
<td>4 (31)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ST78-MRSA-IV[2B]</td>
<td>12</td>
<td>2 (17)</td>
<td>4 (33)</td>
<td>5 (42)</td>
<td>0</td>
<td>1 (8)</td>
<td>5 (3 CAP, 1 SSTI, 1 MSI)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>ST93-MSSA</td>
<td>10</td>
<td>3 (30)</td>
<td>5 (50)</td>
<td>2 (20)</td>
<td>0</td>
<td>0</td>
<td>3 (1 SSTI, 1 MSI, 1 CAP)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>ST45-MSSA</td>
<td>7</td>
<td>1 (14)</td>
<td>4 (57)</td>
<td>1 (14)</td>
<td>1 (14)</td>
<td>0</td>
<td>5 (2 MSI, 1 SSTI, 1 CAP, 1 IE)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>ST15-MSSA</td>
<td>6</td>
<td>2 (33)</td>
<td>4 (67)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (1 SSTI, 1 MSI)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>ST22-MRSA-IV[2B]</td>
<td>5</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>0</td>
<td>2 (40)</td>
<td>2 (1 SSTI, 1 MSI)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>ST5-MRSA-IV[2B]</td>
<td>5</td>
<td>1 (20)</td>
<td>4 (80)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (1 MSI)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>ST47-MSSA</td>
<td>5</td>
<td>0</td>
<td>3 (60)</td>
<td>0</td>
<td>2 (40)</td>
<td>0</td>
<td>4 (2 MSI, 2 IE)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>ST30-MRSA-IV[2B]</td>
<td>4</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (1 SSTI, 1 CAP)</td>
<td>2 (50)</td>
</tr>
</tbody>
</table>

Values are number (%) unless otherwise stated.

Abbreviations: BSI, bloodstream infection; CAP, community acquired pneumonia; IE, infective endocarditis; MSI, musculoskeletal infection; SSTI, skin and soft tissue infection.

### Table 3. *S. aureus* Clones Ranked by Illness Severity Score (ISS) and Individual Markers of Severity

<table>
<thead>
<tr>
<th>Rank</th>
<th>Clone</th>
<th>Mean ISS</th>
<th>Mean SAPS II Score [rank]</th>
<th>30-day Mortality (%) [rank]</th>
<th>Requirement for ICU/HDA (%) [rank]</th>
<th>Proportion BSI (%) [rank]</th>
<th>In Hospital LOS [rank]</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>ST15-MSSA</td>
<td>−0.411</td>
<td>12.7 [9]</td>
<td>0/6 (0) [7]</td>
<td>0/6 (0) [7]</td>
<td>2/6 (33) [6]</td>
<td>2.05 [8]</td>
</tr>
<tr>
<td>9</td>
<td>ST5-MRSA-IV[2B]</td>
<td>−0.528</td>
<td>14.6 [8]</td>
<td>0/5 (0) [7]</td>
<td>0/5 (0) [7]</td>
<td>1/5 (20) [9]</td>
<td>2.72 [5]</td>
</tr>
<tr>
<td>10</td>
<td>ST93-MRSA-IV[2B]</td>
<td>−0.655</td>
<td>9.2 [10]</td>
<td>0/13 (0) [7]</td>
<td>0/13 (0) [7]</td>
<td>0/13 (0) [10]</td>
<td>1.57 [9]</td>
</tr>
</tbody>
</table>

Abbreviations: BSI, bloodstream infection; HDA, high-dependency area; ICU, intensive care unit; ln, natural log; LOS, length of stay; SAPS II, Simplified Acute Physiology Score II.
clone (Table 5) with the exception of a smaller proportion with a high-ranking ISS clone having been hospitalized in the last 12 months (23% vs 47%; \( P = .04 \)).

**DISCUSSION**

By combining objective clinical markers of illness severity (30-day mortality, requirement for intensive hospital support, proportion associated with bacteremia and LOS) using PCA, we were able to rank clones according to severity of illness and subsequently compare this with their corresponding DNA-based genotype in addition to host factors to provide information about the possible roles the multitude of different virulence determinants found played. To our knowledge, this is the first time such an attempt has been made to rank different invasive *S. aureus* clones with the aid of statistical methods. Based on their mean ISS, the CC45-MSSA and ST30-MRSA-IV[2B] clones caused the most severe invasive infections, a finding supported by a recent study that found CC30 clones caused the majority of cases of persistent MRSA bacteremia [31].

We expected that a similar rank order of clones might be observed when mean SAPS II scores for the 10 most common clones were compared, given that this score is a well-validated measure of probability of dying while in hospital [22]. The 12 physiological variables (in addition to age, type of admission, and 3 possible comorbidities) that are required for this score led to a different ranking of clones. ST22-MRSA-IV[2B] ranked the highest, likely because of the high median age of patients infected with this clone (80 years) and their associated underlying comorbidities that may have influenced the physiological variables. However, we did observe that 4 out of the 5 top- and bottom-ranking clones based on their mean ISS remained in the top 5 and bottom 5, respectively, for mean SAPS II score.

Analysis of the microarray-based genotype of the 10 most common clones revealed a number of observations. The *agr* type of the highest-ranking clones belonged to types I and III,
and 8/10 clones had capsule 8 detected, in keeping with another study that found these features predictive of persistent bacteremia [31]. The virulence determinant profiles of particular cMRSA and cMSSA clones in this study closely matched those reported by other groups studying the same S. aureus clones [29, 32–34], suggesting that virulence profiles among the different clones are relatively stable.

While all the S. aureus clones in this study had genes for several adhesion factors, all but 1 of the top 5 clones compared with none of the lower 5 clones carried the cna gene, which has been reported to be important in invasive MSI and IE [9, 35] and more common in isolates causing persistent bacteremia [31].

An association between the number and type of enterotoxin genes detected and the severity of infection was also seen. Four of the 5 highest ISS-ranking S. aureus clones all contained the egc compared with only 1 of the bottom 5, suggesting that this cluster may be important for virulence. Neutralizing antibodies to egc SAgs are found only in the minority of individuals [36], yet these SAgs elicit strong proinflammatory cytokine responses in vitro and are excreted mainly in the exponential phase of growth in contrast to other SAgs that are usually expressed in the postexponential phase [36, 37]. It is possible that severe invasive infection with egc-containing clones may be in part due to inefficient adaptive immune responses to egc [37], and in at least 1 case of PVL-negative ST45-MSSA (necrotizing fasciitis requiring amputation), virulence was attributed to the presence of the egc [11].

Other enterotoxins were found less consistently. For example, sed + sej + ser found in 1 of the top-ranking ST47-MSSA clones has previously been found significantly more often in BSI isolates [38] and invasive isolates [39], while sec + sel found in the majority of the CC45-MSSA (ranks 1 and 3), ST22-MRSA-IV[2B] (rank 5), and ST78-MRSA-IV[2B] (rank 6) isolates may also contribute to their virulence with supporting evidence found from 3 lethal cases of S. aureus purpura fulminans harboring the sec gene [10]. The finding that enterotoxins other than those contained in the egc were not always detected within clonal complexes was not surprising, as these genes are carried on plasmids or pathogenicity islands and so are prone to horizontal gene transfer, a finding observed in other studies [12, 39].

In contrast, there was no clear association between a clone’s mean ISS and the presence of genes encoding PVL, with PVL-positive clones ranked second, fourth and 10th, rather than being associated only with the highest-ranking clones, as would be expected if they were uniformly associated with more severe disease. This finding is supported by other studies that found there was no difference in severity of illness caused by PVL-positive and -negative strains of MRSA [40, 41].

We also observed that cMSSA clones caused more severe disease than cMRSA clones, with 3 of the 4 highest-ranking S. aureus clones being methicillin susceptible, in contrast to 5 of the 6 lowest-ranking clones being methicillin resistant. In addition, the mean ISS for all MSSA clones was higher than that of MRSA clones. Of particular interest in this respect was the finding that ST93-MSSA ranked higher (fourth-highest ISS) compared with ST93-MRSA-IV[2B] (10th). These data support the proposal that β-lactam resistance in S. aureus may result in a fitness cost. It has recently been shown that MSSA cells have a shorter generation time, resulting in a higher cell count within an hour compared with MRSA cells; and a higher number of MRSA cells are required, compared with MSSA cells, to cause the same death rate in mice [16]. Alternatively, a differential expression of genes such as the egc may explain some of this difference, as egc was detected in a greater number of MSSA isolates (19/42, 45%) compared with MRSA isolates (9/54, 17%). Undetected virulence determinants in MSSA (that are absent in MRSA) may explain the differential values of the ISS for the enterotoxin-negative ST93-MSSA and ST93-MRSA-IV[2B].

Finally, somewhat unexpectedly, host factors such as age or diabetes did not appear to be associated with the ISS. Recent hospitalization was associated with infection with lower-ranking clones, suggesting that despite the tendency for older patients and those with significant comorbidities to have greater rates of recent hospitalization, the effect of these factors on outcome are potentially outweighed by a clones’ intrinsic virulence. This provides further supportive evidence that true “community-acquired” S. aureus clones may be more virulent than those acquired during hospitalization [42]. While the impact of appropriate antimicrobial therapy on clinical outcomes was not assessed in this study, it is likely that the delay in appropriate therapy we observed for the MRSA group in a previous study [17] would not alter the above findings.

The strengths of this study include the prospective design, the extent of clinical information collected, and extensive molecular characterization of isolates that caused infection. Limitations of this study include the fact that it was conducted in one relatively isolated Australian city, and therefore may not represent the clinical and molecular epidemiology of invasive S. aureus infection in other regions; the DNA microarray employed in this study detected only those genes encoding for various resistance and virulence determinants and not the degree of expression of the genes in vivo; and finally, other genes that may play a key role in the virulence of certain S. aureus strains such as α-type phenol-soluble modulins (PSMs) [43] were not represented on the DNA microarray.

The diversity of ISS values for S. aureus clones and the apparent association with key virulence genes such as cna and the egc support the mounting evidence that “not all S. aureus strains are created equal” [42] and that virulence is not determined by any single determinant (eg, PVL) but a multitude of interacting virulence determinants [39, 44, 45]. Whilst our
proposed ISS requires further validation in larger studies, virulence profile data may ultimately influence treatment of patients with invasive S. aureus infection (eg, the use of ribosomal active agents for toxin producers [46], intravenous immunoglobulin [10], and specific anti-SAg agents [47]), in addition to guiding S. aureus vaccine development and further refining infection control policies targeting those S. aureus clones that are associated with the potential for the most morbidity and mortality.

Notes

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