Factors Influencing Time to Vancomycin-Induced Clearance of Nonendocarditis Methicillin-Resistant Staphylococcus aureus Bacteremia: Role of Platelet Microbicidal Protein Killing and \textit{agr} Genotypes

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\textbf{Background.} Vancomycin susceptibility, the accessory gene global regulator (\textit{agr}) genotype and function, staphylococcal cassette chromosome (SCC) \textit{mec} type, and susceptibility to cationic thrombin-induced platelet microbicidal protein 1 (tPMP-1) have been individually predictive of duration of methicillin-resistant \textit{Staphylococcus aureus} (MRSA) bacteremia. This investigation evaluated the interrelationship of these factors with time to clearance of MRSA bacteremia during vancomycin therapy in patients without endocarditis.

\textbf{Methods.} Vancomycin minimum inhibitory concentration and in vitro killing, \textit{agr} function (\textit{\delta}-hemolysin activity), \textit{agr} group, SCC\textit{mec} type, and survival in tPMP-1 killing assays were determined for 29 MRSA bacteremia isolates.

\textbf{Results.} Increased resistance to tPMP-1 killing was observed with \textit{agr} group III MRSA \textit{P} \leq .025 and MRSA with reduced or absent \textit{agr} function \textit{(P} = .023). The median time to clearance of MRSA bacteremia was earlier for \textit{agr} group III (3 days) versus group I (10.5 days) or II (15 days) \textit{(P} = .001). In multivariate analysis, \textit{agr} group II, reduced tPMP-1 killing in vitro, and prior vancomycin exposure were significant independent predictors of longer MRSA bacteremia duration.

\textbf{Conclusions.} Specific genotypic, phenotypic, and clinical parameters appear to correlate with persistent MRSA bacteremia. The interrelationship of these and other factors probably contributes to vancomycin-mediated clearance of MRSA bacteremia.

Several retrospective studies have independently found that methicillin-resistant \textit{Staphylococcus aureus} (MRSA) strains with vancomycin minimum inhibitory concentrations (MICs) at the upper limits of microbiological susceptibility (vancomycin MIC, 1–2 mg/L) are associated with inferior vancomycin treatment outcomes in pneumonia and bacteremia \cite{1–4}. More recently, bacteremia caused by MRSA with a vancomycin MIC of 2 mg/L was associated with significantly higher mortality when treated empirically with vancomycin than bacteremia caused by MRSA with a vancomycin MIC of 1 mg/L \cite{5, 6}. Bacteremia with such vancomycin-“susceptible” isolates exhibiting significant proportions...
of resistant subpopulations (heterogeneous vancomycin-intermediate \textit{S. aureus} [hVISA]) has also been associated with treatment failure and longer time to clearance of bacteremia [7], and higher hVISA rates have also been associated with higher vancomycin MICs in MRSA [8]. Importantly, a recent study has suggested a paradoxically inverse relationship between vancomycin MIC and clinical outcome [9]. Thus, the relative contributions of pertinent phenotypic traits to prolonged MRSA bacteremia have not been completely defined.

The accessory gene regulator (\textit{agr}) locus in \textit{S. aureus} establishes a quorum-sensing mechanism that regulates expression of dozens of virulence and housekeeping genes in a growth phase–dependent manner. In general, increased \textit{agr} expression increases secreted virulence factors (eg, \alpha-toxin) and decreases cell-associated surface adhesins (eg, microbial surface components recognizing adhesive matrix molecules [MSCRAMMs]) [10]. The \textit{agr} locus is polymorphic, defining 4 major \textit{S. aureus} \textit{agr} groups (I, II, III, IV), on the basis of structure of the auto-inducing peptide encoded by this operon. The majority of the early vancomycin-intermediate \textit{S. aureus} strains in the United States belonged to \textit{agr} group II, with many of these strains also showing loss of \textit{agr} function accompanying reduced vancomycin susceptibility. Follow-up studies demonstrated that the \textit{agr} group II genotype and attenuated \textit{agr} function were associated with reduced vancomycin treatment success [1] and prolonged bacteremia [11]. A subsequent study showed a pharmacodynamic trend toward development of reduced glycopeptide susceptibility with subinhibitory vancomycin exposure in an \textit{agr} group II knockout strain [12]. The exact linkage between \textit{agr} genotype and dysfunction with prolonged MRSA bacteremia remains to be delineated.

Platelet microbicidal proteins (PMPs) are a family of cationic antimicrobial peptides that are important in host defense against the establishment and/or progression of endovascular infections in humans and in experimental endocarditis models [13–17]. Bacteria exhibiting reduced susceptibility in vitro to thrombin-induced PMP-1 (tPMP-1), especially \textit{S. aureus}, have been associated with prolonged bacteremia in patients [11]. However, the exact relationship among tPMP-1 activity, distinct MRSA genotypes, and vancomycin susceptibility profiles is not known.

The present study employed a set of well-characterized MRSA bacteremia isolates obtained from multiple medical centers throughout the United States to avoid clonal issues and geographic biases. We used this collection to evaluate potential interrelationships among tPMP-1 susceptibility, \textit{agr} groups and function, staphylococcal cassette chromosome (SCC) mec type, vancomycin susceptibility in vitro, and vancomycin-induced time to clearance of MRSA bacteremia. We focused exclusively on patients without endocarditis to avoid the potentially confounding aspects of evaluating duration of bacteremia in settings where metastatic abscess formation and cardiac and other surgical interventions play such an integral role in microbiological and clinical outcomes.

**METHODS**

**Bacterial strains and their clinical background.** Twenty-nine microbiologically confirmed MRSA bloodstream isolates from 29 unique patients at multiple medical centers (1998–2002) were used in this investigation. Isolates were initially obtained as part of 4 multicenter prospective clinical trials evaluating vancomycin versus comparators in patients with MRSA bacteremia: (1) a phase 3 study of quinupristin-dalfopristin versus vancomycin for catheter-related bacteremia, (2) a phase 3 study of linezolid versus vancomycin for methicillin-resistant \textit{Staphylococcus} species infections, (3) a phase 3b MRSA study of “high-dose” vancomycin versus “conventionally dosed” vancomycin plus quinupristin-dalfopristin, and (4) a phase 4 National Nosocomial Resistance Surveillance Group study. For each study, appropriate ethical regulations were followed, and the study was approved by the ethics committee or institutional review board at each participating institution. Strains from patients who received vancomycin therapy were collected randomly by one of the authors (G.S.) as a component of other investigations [2, 4]. The 29 specific MRSA isolates assayed in this study were selected to provide a statistically relevant and representative range of vancomycin-induced bloodstream clearance durations, and this number of isolates was calculated to provide adequate statistical power to our analyses.

Daily blood cultures were obtained until bacteremia clearance in all patients, and only the initial isolate from each patient was studied. None of the patients had confirmed endocarditis, osteomyelitis, or other complicated infection, and none were neutropenic. The absence of such invasive MRSA syndromes was determined by standard clinical and radiographic queries. Patients were categorized by the presence or absence of prior intravenous vancomycin exposure in the 30 days before development of MRSA bacteremia. Patients who received $\geq$1 dose of vancomycin within the 30 days before the initial MRSA positive blood culture were classified in the “previous vancomycin use” group. Of the 12 patients who had received vancomycin in the preceding 30 days, 7 patients had a prior MRSA infection, 4 had received 1 dose for prophylaxis, and 1 had received 3 days of treatment for empiric coverage of a gram-positive infection that did not turn out to be MRSA. The “no previous vancomycin use” group included those patients who had received no vancomycin within 30 days before the initial MRSA positive blood culture. Vancomycin use during the 30 days before presentation with MRSA bacteremia was identified by chart review, patient interview, and discussion with the patient’s primary care physician and other physicians identified as having provided care during that time period. Isolates were
stored at −70°C and cultured and maintained on trypticase-soy blood agar. Vancomycin dose strategies and targeted trough levels were selected by the primary treating physician.

**Genotypic and phenotypic assays.** Multiplex PCR was used to characterize mec cassettes and to characterize agr groups, as described elsewhere [18–20]. A semiquantitative δ-hemolysin functional assay was performed to assess and score agr function (from 0 to +++) [21]. Vancomycin MICs were determined using Clinical and Laboratory Standards Institute broth microdilution methods [22] and E-test (AB Biodisk), under conditions suggested by the manufacturer. American Type Culture Collection 29213 (S. aureus) and 33591 (MRSA) reference strains were used as internal controls. In vitro vancomycin killing assays were performed in duplicate with use of a starting inoculum of ~1 × 10^8 colony-forming units (CFU)/mL in Mueller-Hinton broth containing vancomycin at 16 mg/L. The vancomycin concentration was selected to represent target trough serum levels recommended for treatment of bacteremic S. aureus infection [23]. Bactericidal activity was defined as a reduction of ≥3 log_{10} CFU/mL, compared with the starting inoculum. The presence of the hVISA phenotype was determined using the glycopeptide resistance detection E-test [24].

Among the PMP family of peptides, tPMP-1 and its homologues appear to be the most abundant in mammalian platelets [25]. This peptide is orthologous to the major variant of platelet factor 4 in humans [26]. The percentage survival of stationary-phase MRSA was determined in a killing assay using tPMP-1 from rabbits, prepared and standardized for bioactivity as described elsewhere [17]. Bacterial densities were adjusted spectrophotometrically to 1 × 10^8 CFU/mL (optical density at 600 nm, 0.5), serially diluted to 1 × 10^7 CFU/mL, and added to tPMP-1 (1 or 2 mg/L). Viable cell counts were obtained at time 0 and after 120 min incubation at 37°C by plating serial dilutions of the assay suspension on sheep blood agar plates. Colonies were counted after 24-h incubation at 37°C. Assays were performed in duplicate, and the mean percentage survival at 2 h (± standard deviation) was analyzed statistically.

**Outcomes.** The primary study end points were (1) statistically validated correlates among ≥1 of the following variables: (genotypic) agr group, SCCmec type, (phenotypic) δ-hemolysin expression level (surrogate for agr function), tPMP-1 susceptibility, and vancomycin MIC; and (2) statistically validated correlates among time to clearance of MRSA bacteremia (days) and ≥1 of the preceding genotypic and phenotypic parameters. Time to bacteremia clearance was defined as the time, in days, from the initiation of vancomycin therapy until the first day with negative blood cultures after the last positive culture. To eliminate investigator bias, all outcomes and clinical information were determined and documented before in vitro testing, and all in vitro testing was performed by investigators blinded to the clinical and outcome data.

**Statistical analyses.** Continuous and ordinal variables were compared using Kruskal-Wallis analysis of variance and Mann-Whitney U tests. Categorical variables were compared using χ^2 tests or Fisher’s exact tests, where appropriate. Correlation analysis was used to examine the relationships between MRSA genotypes and phenotypes. We used classification and regression tree modeling, a form of binary recursive partitioning, to identify break points in the percentage of S. aureus survival in PMP assays for analysis of vancomycin response. Tree-based models are useful for both classification and regression problems. This type of modeling identifies which dichotomous split on which predictor variable will maximally improve the predictability of the dependent variable. The predictor variable(s) may be a mixture of nominal and/or ordinal scales. The dependent variable may be quantitative or qualitative (ie, nominal or categorical). The regression trees parallel regression analysis. Finally, median time to bloodstream MRSA clearance was analyzed using univariate (Kaplan-Meier and log rank test) and multivariate (Cox proportional hazards) analyses. Differences were considered to be statistically significant at P<.05. All analyses were performed using Systat 11 software (Systat Software).

**RESULTS**

**Clinical and microbiological profiles.** Relevant patient demographic characteristics and comorbid conditions for the 29 cases of MRSA bacteremia are listed in Table 1. Of the 19 cases of non–catheter-related bacteremia, 15 emanated from soft-tissue abscesses. Characteristics of the corresponding MRSA organisms are shown in Table 2, including agr genotype, SCCmec type, agr functional status, and in vitro vancomycin susceptibility. Bacteremia was catheter related in 10 cases and non–catheter related in 19. The MRSA isolates were predominantly characterized by the following features conventionally associated with “health care onset” rather than “community onset”: SCCmec II (93%), agr group II (65%), agr dysfunction (76%), and elevated vancomycin MIC (2 mg/L in 48% of SCCmec II MRSA isolates). Twenty-seven patients (93%) underwent echocardiography; no patients had echocardiographic evidence of vegetative endocarditis.

**Catheter-related versus non–catheter-related bacteremia.** We hypothesized that the duration of bacteremia would be shorter in catheter-related cases, given this easily identifiable and removable focus. Thus, the relevant study variables were also evaluated on the basis of catheter or noncatheter source status. As predicted, catheter-related bacteremia was confirmed to be of significantly shorter duration than non–catheter related bacteremia (6 vs >10 days; P = .021). No differences were noted between MRSA in catheter-related versus non–catheter related bacteremia with respect to vancomycin MIC (P = .360), vancomycin killing in vitro (P = .251), tPMP-1 susceptibility (P = .748), agr functional status (P = .593), agr genotype...
Table 1. Baseline Clinical and Demographic Characteristics of 29 Patients with Non-endo- 
carditis Methicillin-Resistant Staphylococcus aureus Bacteremia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Age, mean years ± SD (median)</td>
<td>68 ± 17 (73)</td>
</tr>
<tr>
<td>Male sex</td>
<td>18 (62)</td>
</tr>
<tr>
<td>ICU at baseline</td>
<td>14 (48)</td>
</tr>
<tr>
<td>CrCl, mean mL/min ± SD (median)</td>
<td>61 ± 35 (58)</td>
</tr>
<tr>
<td>Select underlying diseases</td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>12 (41)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>10 (34)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>9 (31)</td>
</tr>
<tr>
<td>Steroids</td>
<td>8 (28)</td>
</tr>
<tr>
<td>ESRD with HD</td>
<td>6 (21)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>5 (17)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>5 (17)</td>
</tr>
<tr>
<td>Transplantation</td>
<td>1 (3)</td>
</tr>
<tr>
<td>HIV infection</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Source of bacteremia</td>
<td></td>
</tr>
<tr>
<td>Catheter related</td>
<td>10 (34)</td>
</tr>
</tbody>
</table>
| Non-catheter related (non- 
endocarditis)                         | 19 (66)             |
| Prior vancomycin treatment within 30 days of first 
culture                           | 12 (41)             |
| Onseta                                |                     |
| Community                             | 7 (24)              |
| Health care                           | 22 (76)             |
| Vancomycin steady state trough, mean mg/L ± SD (median) | 14 ± 5 (12) |

**NOTE.** Data are no (%) of patients, unless otherwise indicated. CrCl, creatinine clearance; ESRD, end-stage renal disease; HD, hemodialysis; HIV, human immunodeficiency virus; ICU, intensive care unit; SD, standard deviation.

a Onset of infection was defined based on criteria developed by the Centers for Disease Control and Prevention. Health care-onset methicillin-resistant S. aureus was identified by >1 of the following patient criteria: (1) culture obtained >48 h after admission with history of S. aureus infection or colonization; (2) previous hospitalization within 1 year; (3) admission to nursing home, skilled nursing facility, or hospice; (4) history of dialysis or surgery; or (5) permanent indwelling catheter or medical device. Community-onset methicillin-resistant S. aureus was defined epidemiologically according to the following criteria: (1) culture obtained in the outpatient setting or within 48 h of hospital admission and (2) no health care–associated risks (eg, hospitalization or surgery within 1 year, admission to nursing home, skilled nursing facility or hospice; and hemodialysis).

(P = .779), or SCCmec type (P = .632) (see detailed analyses below).

**hVISA cases.** Three (10.3%) of 29 cases were caused by MRSA with hVISA phenotypes, as detected by glycopeptide-

 resistance detection. The vancomycin MIC was 2 mg/L in 2 isolates and 1 mg/L in the third.

**Relationship between tPMP-1 susceptibility, agr group and function, SCCmec type, and in vitro vancomycin susceptibility.** There was a significant relationship between the survival of MRSA strains at 1- versus 2-mg/L tPMP-1 (Spearman ρ, 0.880; P < .001). Moreover, there was a significant correlation between tPMP-1 efficacy at 1 and 2 mg/L and both agr group (P = .025) and agr function (δ-hemolysin activity) (P = .023). Specifically, agr group III MRSA and MRSA with reduced or absent δ-hemolysin activity were significantly more resistant to tPMP-1 killing (median survival, 82% vs 16% for agr group III vs non-group III MRSA; P = .002).

The agr group was related to agr function (P = .008) and SCCmec genotype (P = .019). Group II MRSA demonstrated reduced agr function at a higher frequency than other agr groups. The agr function was also related to SCCmec genotype (P = .005) and prior vancomycin use (P = .008), with reduced agr function more common in SCCmec type II and with prior vancomycin exposure.

Vancomycin MIC was significantly related to both agr function (Spearman ρ = −0.556; P = .004) and prior vancomycin use (P < .001). MRSA with attenuated agr function (δ-hemolysin score, 0–1) had significantly higher vancomycin MICs (P = .006) (Table 2). Forty-eight percent of SCCmec type II MRSA isolates had vancomycin MICs of 2 mg/L, compared with none of the SCCmec type IV MRSA isolates (P = .186).

No significant relationship was noted between vancomycin MIC and the percentage survival of MRSA on exposure to tPMP-1 under either assay condition (1 or 2 mg/L peptide). The relationship between higher vancomycin MIC and reduced vancomycin killing was of borderline significance (Spearman ρ = −0.307; P = .062). Reduced killing activity of vancomycin in vitro was significantly related to prior vancomycin exposure (P = .011).

**Relationship between MRSA genotype and phenotype with time to clearance of MRSA bacteremia.** The median time to clearance of bacteremia was significantly shorter for agr group III (3 days) than for either agr group I (10.5 days) or agr group II (15 days) MRSA (P = .001). Kaplan-Meier analysis confirmed that agr group was significantly associated with time to bacteremia clearance (Figure 1). In the Cox proportional hazards model, vancomycin in vitro killing of MRSA was also independently associated with time to clearance of MRSA bacteremia. Thus, MRSA isolates demonstrating a reduction of <3.0 log_{10} CFU/mL at 24 h in vitro were more likely to cause prolonged bacteremia clinically (median time to clearance, >10.5 days) than isolates with a reduction of ≥3.0 log_{10} CFU/mL (median time to clearance, 4 days; P = .001) (Figure 2).

The median time to clearance of bacteremia with vancomycin
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therapy was longer for MRSA strains with a vancomycin MIC of 2 mg/L than for those with an MIC of <2 mg/L (>9.0 vs 6.5 days; \(P = .067\)). The median time to bacterial bloodstream clearance was 10 days with deficient or absent \(agr\) function \((\delta\)-hemolysin score, 0–1), compared with 6.5 days for MRSA with higher \(agr\) function \((\delta\)-hemolysin score, 2–4) \((P = .274)\). No significant differences in time to bacteremia clearances were noted between the SCCmec types II and IV (median time to eradication, 10.5 vs 6.5 days; \(P = .773\)).

Tree-based modeling demonstrated that a 15% MRSA survival after tPMP-1 exposure at 1 mg/L in vitro was a categorical break point for eradicating MRSA bacteremia with vancomycin therapy. Among patients with MRSA exhibiting <15% survival after tPMP-1, bacterial eradication was achieved with vancomycin therapy in 5 (83%) of 6 patients, compared with only 9 (39%) of 23 with ≥15% survival after tPMP-1 \((P = .054)\). The median time to clearance of bacteremia for MRSA isolates demonstrating <15% survival \((n = 6)\) was 5.5 days, compared with >15 days for those demonstrating ≥15% survival \((n = 23; \ P = .046)\) (Figure 3).

Kaplan-Meier analysis of time to clearance of MRSA bacteremia by previous vancomycin use is depicted in Figure 4, showing a significantly longer time to clearance in patients with previous vancomycin exposure \((n = 12)\) than in vancomycin-naïve patients \((n = 17; \ P = .020)\). Time to catheter removal did not differ between these groups.

Clinical factors present in the database in sufficient numbers to confound the analysis of duration of bacteremia (patient age, intensive care unit placement, diabetes, glucocorticoid use, and hemodialysis) were stratified against the following variables that were found to be associated with prolonged bacteremia: \(agr\) group, prior vancomycin exposure, and vancomycin killing in vitro. No relevant clinical factors were associated with \(agr\) group

![Figure 1](https://academic.oup.com/jid/article-abstract/201/2/233/2192255)

**Figure 1.** Median time to clearance of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia by accessory gene global regulator \((agr)\) specificity group. The median time to clearance was earlier for \(agr\) group III MRSA (3 days) \((dashed\ line)\) than for group I (10.5 days) \((dotted\ line)\) or group II (15 days) \((solid\ line)\) MRSA \((P = .001)\).

**Table 2. Genotypic and Phenotypic Characteristics of *Staphylococcus aureus* (MRSA) Bloodstream Isolates by Vancomycin Minimum Inhibitory Concentration (MIC)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All isolates ((n = 29))</th>
<th>MIC 0.5 mg/L ((n = 10))</th>
<th>MIC 1.0 mg/L ((n = 6))</th>
<th>MIC 2.0 mg/L ((n = 13))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(agr) group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6 (21)</td>
<td>2 (20)</td>
<td>2 (33)</td>
<td>2 (15)</td>
<td>NS*</td>
</tr>
<tr>
<td>II</td>
<td>19 (66)</td>
<td>5 (50)</td>
<td>4 (67)</td>
<td>10 (77)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4 (14)</td>
<td>3 (30)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td></td>
</tr>
<tr>
<td>SCCmec type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>27 (93)</td>
<td>9 (90)</td>
<td>5 (83)</td>
<td>13 (100)</td>
<td>.306</td>
</tr>
<tr>
<td>IV</td>
<td>2 (7)</td>
<td>1 (10)</td>
<td>1 (17)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>(agr) function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preserved</td>
<td>7 (24)</td>
<td>5 (50)</td>
<td>2 (33)</td>
<td>0 (0)</td>
<td>.006</td>
</tr>
<tr>
<td>Reduced or absent</td>
<td>22 (76)</td>
<td>5 (50)</td>
<td>4 (67)</td>
<td>13 (100)</td>
<td></td>
</tr>
<tr>
<td>Survival with tPMP-1 in vitro, mean % ± SD (median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/L</td>
<td>36 ± 29 (25)</td>
<td>42 ± 31 (32)</td>
<td>26 ± 18 (22)</td>
<td>35 ± 32 (23)</td>
<td>.727</td>
</tr>
<tr>
<td>2 mg/L</td>
<td>24 ± 29 (10)</td>
<td>31 ± 33.4 (17)</td>
<td>14 ± 15 (6)</td>
<td>23 ± 32 (8)</td>
<td>.636</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no (%) of isolates, unless otherwise indicated. \(agr\), accessory gene regulator; NS, not significant; SCC, staphylococcal cassette chromosome; SD, standard deviation; tPMP-1, thrombin-induced platelet microbicidal protein 1.

*\(agr\) group I versus II, \(P = .491\); \(agr\) group I versus III, \(P = .385\); \(agr\) group II versus III, \(P = .145\).
Figure 2. Kaplan-Meier analysis of time to clearance of methicillin-resistant Staphylococcus aureus (MRSA) bacteremia by vancomycin in vitro killing activity. Upper (dotted) line represents 16 patients whose MRSA isolates demonstrated a reduction of $<3.0 \log_{10}$ colony-forming units per mL at 24 h; lower (solid) line represents 13 patients whose MRSA isolates demonstrated a reduction of $\geq 3.0 \log_{10}$ colony-forming units per mL ($P = .001$).

Figure 3. Kaplan-Meier analysis of time to clearance of methicillin-resistant S. aureus (MRSA) bacteremia by thrombin-induced platelet microbicidal protein 1 (tPMP-1) activity. Upper (dotted) line represents 23 patients whose MRSA isolates demonstrated $\geq 15\%$ MRSA survival at 2 h with exposure to 1 mg/L tPMP-1; lower (solid) line represents 6 patients whose MRSA isolates demonstrated $<15\%$ MRSA survival for the same exposure ($P = .046$).

II, vancomycin killing, or PMP susceptibility. We found prior vancomycin use to be significantly more common among patients undergoing hemodialysis ($P = .019$).

**DISCUSSION**

Increased vancomycin MICs within the susceptible range, reduced vancomycin killing in vitro, the agr group II genotype, reduced or absent agr function, and resistance to killing in vitro by mammalian cationic host defense peptides have all been associated to varying degrees with prolonged MRSA bacteremia [1–7, 11, 13–17, 27]. Although some of these characteristics have been examined in cases of S. aureus endocarditis [28], to our knowledge none of the prior studies has examined these pathogen-specific factors collectively in a well-characterized, geographically diverse strain set from patients without endocarditis to determine their interrelations statistically. Furthermore, the effect of prior vancomycin exposure on these properties of MRSA (because of its potential impact on MRSA microbiological characteristics, especially vancomycin MICs [29, 30]) has not been evaluated elsewhere.

The current analysis of non-endocarditis MRSA bacteremia cases revealed several MRSA properties that were associated with prolonged bacteremia and were highly interrelated: (1) susceptibility to tPMP-1 in vitro was related to agr group and function; (2) vancomycin MIC was related to agr function and prior vancomycin use; (3) vancomycin killing activity in vitro was related to prior vancomycin use; and (4) agr group II genotype, increased resistance to tPMP-1 killing in vitro, SCCmec type II, and decreased agr function were associated with prolonged duration of bacteremia during vancomycin therapy. Moreover, there was a strong trend toward reduced vancomycin killing of MRSA with higher vancomycin MIC, although this did not achieve statistical significance. Kaplan-Meier analyses demonstrated that agr group II, increased survival with in vitro tPMP-1 exposure, and prior clinical vancomycin exposure were independent predictors of the persistence of MRSA bacteremia.

Prior work relating S. aureus tPMP-1 susceptibility profiles in vitro to specific clinical disease syndromes demonstrated that a relative “break point” of $>40\%$ survival was associated with endocarditis [31]. Conversely, isolates with $<40\%$ survival were frequently associated with bacteremic soft-tissue abscesses [31]. In the current study of strictly non-endocarditis bloodstream isolates, tree-based modeling showed that relative resistance to tPMP-1 killing in vitro ($\geq 15\%$ survival) is significantly related to prolonged MRSA bacteremia. The exclusion of endocarditis isolates in this study probably explains the relatively low break point ($\geq 15\%$ survival) distinguishing bacteremic duration groups, compared with previous studies. It is interesting that a recent study comparing MRSA strains from persistent versus resolving MRSA bacteremia associated the agr group III genotype (ie, predicted clonal complex 30) and increased survival in tPMP-1 assays with the persistent bacteremia group [28]. This latter study differed from the current one in that it was...
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4-day gentamicin with daptomycin therapy for the treatment of MRSA bacteremia and endocarditis [32]. In this latter study, the isolates had lower vancomycin MIC and were more likely to have preserved agr function and be agr group I. Clearly, differences in MRSA characteristics between different trial isolates may reflect the variability in strain selection by study center, clinical features (ie, enrollment criteria), and/or time periods of enrollment. Although the present study analyzed MRSA isolates from multiple medical centers, it was limited by its small sample size and retrospective nature. Moreover, target trough levels were not systematically analyzed, so their effects on the duration of bacteremia in individual patients were not determined. Vancomycin trough levels were consistently >15 mg/L in 5 patients, and vancomycin failed to clear the bacteremia in all 5, despite 8–17 days of therapy. This finding raises the suspicion that treatment failure with vancomycin in MRSA bacteremia at a vancomycin MIC of 2 mg/L may reflect more than a pharmacokinetic hurdle. If so, such a relationship may represent, at least in part, differences in organism pathogenicity fostering persistent bacterial infection. Specifically, reduced in vitro susceptibility to vancomycin may portent a reduction in synergistic or additive antibacterial activities between tPMP-1 and vancomycin. Such beneficial interactions between tPMP-1 and oxacillin have been demonstrated elsewhere [15].

In summary, both antimicrobial selection pressure and evolutionary changes driven by advantages in virulence may select for properties in specific MRSA strains that are associated with prolonged bacteremia. Whereas these properties can influence each other, agr group II genotype, enhanced in vitro survival with platelet host defense peptide killing, and prior vancomycin exposure were independent predictors of the persistence of MRSA bacteremia in non-endocarditis cases.

References


