Correlation of vancomycin and daptomycin susceptibility in *Staphylococcus aureus* in reference to accessory gene regulator (agr) polymorphism and function

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**Objectives**: Recently, an association between the accessory gene regulator (agr) in *Staphylococcus aureus* and the development of vancomycin resistance secondary to suboptimal exposure has been demonstrated. We investigated the relationship of vancomycin with and without gentamicin or rifampicin, and daptomycin in the development of resistance in agr groups I and II.

**Methods**: *S. aureus* belonging to agr groups I and II was exposed to varying concentrations of vancomycin and daptomycin simulating an fAUC/MIC of 14–460 and 30–239, respectively, in an in vitro pharmacodynamic model.

**Results**: Vancomycin regimens resulting in fAUC/MIC of 16.1–107.0 resulted in resistance in agr I and agr II knockout strains, whereas regimens resulting in fAUC/MIC of 16.1 resulted in emergence of resistance in agr I- and agr II-positive strains. Overall, agr-null strains demonstrated a higher likelihood of resistance and a greater change in vancomycin susceptibility. The addition of gentamicin and rifampicin to vancomycin at these same exposures prevented the emergence of resistance. At extremely low daptomycin exposures of fAUC/MIC of 22–66, an increase in MIC of 2–3-fold up to a maximum of 0.75 mg/L was observed. However, this was independent of agr group and/or function and still within the susceptible range of daptomycin.

**Conclusions**: The combination of vancomycin with either rifampicin or gentamicin prevented the emergence of vancomycin resistance in agr I and II *S. aureus* isolates. Changes in daptomycin susceptibility were independent of agr group and function. The association between agr and vancomycin resistance in *S. aureus* requires further investigation.

**Keywords**: *S. aureus*, pharmacodynamics, vancomycin resistance

**Introduction**

The accessory gene regulator (agr) of *Staphylococcus aureus* is a global regulator that modulates the expression of numerous virulence factors in a growth phase-dependent manner.\(^1\) Previous studies have demonstrated a relationship between loss of agr function in an agr group II strain and attenuated bactericidal activity. Although agr group II isolates predominate in the hospital, we have documented the incorporation of agr group I strains into this setting.\(^2\) We have noted that all agr groups develop intermediate resistance to vancomycin with subtherapeutic exposures, with a higher propensity for dysfunctional isolates to display
Materials and methods

RN6607 and RN9120, corresponding to agr II-positive and -null, respectively, were obtained from the Network on Antimicrobial Resistance in *S. aureus*. Methicillin-resistant *Staphylococcus aureus* (MRSA) 3436 and 3402, corresponding to agr I-positive and -null clinical isolates, respectively, were obtained from Detroit Receiving Hospital and University Health Center. Powdered vancomycin, gentamicin and rifampicin of analytical grade were commercially purchased (Sigma, St Louis, MO, USA). Daptomycin analytical powder was provided by the manufacturer (Cubist Pharmaceuticals, Lexington, MA, USA).

Methicillin-resistant *Staphylococcus aureus* ATCC 9341.5 For those regimens outside the bioassay range, pharmacokinetics were extrapolated from known regimens. The AUC was determined using the linear trapezoidal method. Differences between regimens in log$_{10}$ cfu/mL at 72 h were determined using analysis of variance with Tukey’s test for multiple comparisons. For all experiments, a *P* value of ≤0.05 was considered statistically significant.

The emergence of resistance was screened for at multiple time points throughout the simulations. Samples were plated on brain heart infusion agar (for vancomycin) or on Mueller–Hinton agar supplemented with 50 mg/L calcium (for daptomycin) containing 3× and 6× MIC. Susceptibility was confirmed by the Etest to detect subsequent changes in MIC.

Results and discussion

Pre-exposure MICs of vancomycin for agr I-positive and -null and agr II-positive and -null isolates were 0.75/1 and 1/1 mg/L, respectively. All isolates were susceptible to daptomycin (MIC 0.25 mg/L). Observed pharmacokinetic parameters (±SD) for vancomycin and daptomycin are shown in Table 1.

The effect of varying vancomycin concentrations on killing and the emergence of resistance in agr-null group II are displayed in Figure 1. A dose–response relationship was noted throughout the 72 h testing period against all strains tested. An fAUC/MIC of 16.1 resulted in the development of resistance, with detection of 4–8-fold changes in the MIC with all strains. The agr-null isolates displayed higher MIC changes at this dose when compared with agr-positive isolates, regardless of agr type with MICs as high as 8 mg/L (8-fold) (Table 1). Only regimens with an fAUC/MIC greater than 107.0 were able to suppress resistance emergence. The agr-positive isolates displayed up to a 4-fold increase in MIC with an fAUC/MIC of 16.1 and 31.2. Verification of resistance was confirmed in the hollow fibre model by reproducing the lowest concentration that resulted in resistance from the PK/PD model. Resistance breakpoints were

Table 1. Vancomycin and daptomycin pharmacokinetic and pharmacodynamic values resulting from various drug exposures over 72 h in agr-null strains

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>fAUC/MIC ($\mu$g/mL)</th>
<th>fC$_{\text{max}}$ (mg/L)</th>
<th>fC$_{\text{min}}$ (mg/L)</th>
<th>MIC values (mg/L) in agr I/II-null strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>24 h</td>
<td>48 h</td>
<td>72 h</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>62.5</td>
<td>16.1 ± 1.4</td>
<td>1.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>1/1</td>
</tr>
<tr>
<td>(every 12 h)</td>
<td>125</td>
<td>31.2 ± 1.4</td>
<td>2.4 ± 0.3</td>
<td>0.7 ± 0.2</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>74.4 ± 3.7</td>
<td>5.9 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>107.0 ± 7.5</td>
<td>9.3 ± 1.0</td>
<td>2.4 ± 0.1</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>165.6 ± 7.8</td>
<td>13.4 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>1/1</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>0.75</td>
<td>22</td>
<td>1</td>
<td>0.125</td>
<td>0.25/0.25</td>
</tr>
<tr>
<td>(every 24 h)</td>
<td>1.5</td>
<td>66</td>
<td>2.48 ± 1.8</td>
<td>0.25</td>
<td>0.25/0.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>132 ± 0.6</td>
<td>4.1 ± 0.4</td>
<td>0.5</td>
<td>0.25/0.25</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>265 ± 0.8</td>
<td>8.9 ± 0.6</td>
<td>1.9 ± 0.54</td>
<td>0.25/0.25</td>
</tr>
</tbody>
</table>

*These values are displayed as means ± SD.
Factors correlating with reduced susceptibility to vancomycin have been verified in other in vivo and in vitro settings. The penetration of vancomycin into sequestered sites of infection such as pneumonia and endocarditis presents a difficult challenge to maintain therapeutic concentrations. Our findings suggest that vancomycin concentrations correlating with an \( \text{fAUC/MIC} \) of 107.0 (\( \text{fC_{min}} \) 2.4 mg/L) or lower did not suppress the emergence of resistance in an \( \text{agr} \) II-null strain. All strains, regardless of \( \text{agr} \) type and function, exhibited resistance with the \( \text{fAUC/MIC} \) 16.1 regimen.

The \( \text{agr} \) group and function has been noted to play an important role in the development of vancomycin heteroresistance. In vitro, hGISA has occurred in \( \text{agr} \) II-null populations exposed to subtherapeutic vancomycin concentrations, and loss of \( \text{agr} \) function may prove advantageous for organism survival. We have demonstrated the tendency of increased vancomycin resistance in all \( \text{agr} \)-null groups. This is important in hospital settings where we have reported that up to 48% of hospital-associated MRSA had defective \( \text{agr} \) function when compared with only 3.5% of community-associated MRSA strains.

In this study, we were able to reduce the emergence of resistance at doses with an \( \text{fAUC/MIC} \geq 16.5 \) and to minimize or eliminate the emergence of vancomycin resistance with the addition of rifampicin or gentamicin. Although controversy exists regarding the risks and benefits of adding gentamicin or rifampicin to vancomycin to improve patient outcome, our results would suggest that these combinations may prevent the emergence of vancomycin resistance secondary to suboptimal exposures in serious infections such as pneumonia, bacteraemia or endocarditis. Suboptimal daptomycin exposures did not result in bacterial killing at or near detection limits when compared with vancomycin alone only with the \( \text{agr} \) I-null strain, displaying a 2-fold increase in MIC (3 mg/L) at 72 h. No MIC changes were detected in the \( \text{agr} \) I- and II-positive and \( \text{agr} \) II-null strains with the addition of rifampicin.

The pharmacodynamic effects of daptomycin exposures of 0.75–6 mg/kg against RN9120 are displayed in Figure 1. Subtherapeutic regimens of 0.75, 1.5 and 3 mg/kg every 24 h (\( \text{fAUC/MIC} \) 22, 66 and 132) demonstrated early bactericidal kill followed by considerable regrowth in all isolates tested. Minimal regrowth was noted with 6 mg/kg every 24 h (\( \text{fAUC/MIC} \) 265). Increased MIC values were observed at suboptimal doses irrespective of \( \text{agr} \) type or function. Regimens with an \( \text{fAUC/MIC} \) of 22 and 66 resulted in an MIC value of 0.75 and 0.5 mg/L (3- and 2-fold increase, respectively, in the \( \text{agr} \) II-null strain. Similar results were noted with these regimens in the other isolates. All isolates recovered were susceptible to daptomycin (MIC ≤ 1 mg/L). In the hollow fibre model, the \( \text{fAUC/MIC} \) of 22 had no effect on inoculum reduction, resulting in no changes in the MIC.

Many factors have been associated with vancomycin treatment failures. In one study, higher rates of morbidity were correlated with patients infected with hGISA strains. In addition, these patients were more likely to have a high bacterial load and a low initial vancomycin concentration (trough ≤10 mg/L). Factors correlating with reduced susceptibility to vancomycin have been verified in other in vivo and in vitro settings. The penetration of vancomycin into sequestered sites of infection such as pneumonia and endocarditis presents a difficult challenge to maintain therapeutic concentrations. Our findings suggest that vancomycin concentrations correlating with an \( \text{fAUC/MIC} \) of 107.0 (\( \text{fC_{min}} \) 2.4 mg/L) or lower did not suppress the emergence of resistance in an \( \text{agr} \) II-null strain. All strains, regardless of \( \text{agr} \) type and function, exhibited resistance with the \( \text{fAUC/MIC} \) 16.1 regimen.

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Figure 1. (a) Activity of vancomycin (mean ± SD) alone at simulated regimens over 72 h against an \( \text{agr} \) II-null strain RN 9120 (filled circles, growth control; open circles, \( \text{fAUC/MIC} \) 16.1; filled triangles, \( \text{fAUC/MIC} \) 31.2; open triangles, \( \text{fAUC/MIC} \) 55; filled diamonds, \( \text{fAUC/MIC} \) 119; open squares, 3 × MIC mutants and filled squares, 6 × MIC mutants). (b) Activity of vancomycin (mean ± SD) in combination with gentamicin or rifampicin at simulated regimens over 72 h against an \( \text{agr} \) II-null strain RN 9120 (filled circles, growth control; open circles, \( \text{fAUC/MIC} \) 16.1; filled triangles, \( \text{fAUC/MIC} \) 16.1 + G; open triangles, \( \text{fAUC/MIC} \) 16.1 + R; filled squares, 3 × and 6 × MIC mutants). (c) Activity of daptomycin (mean ± SD) at simulated regimens over 72 h against an \( \text{agr} \) II-null strain (filled circles, growth control; open circles, \( \text{fAUC/MIC} \) 22; filled triangles, \( \text{fAUC/MIC} \) 66; open triangles, \( \text{fAUC/MIC} \) 132; filled squares, \( \text{fAUC/MIC} \) 265 and open squares, 3 × MIC mutants).

Rose et al.
in daptomycin resistance and increased MIC values did not correlate with \textit{agr} group or function, which may be an advantage to the use of daptomycin against these strains.

The relationship between \textit{agr} function and daptomycin was important to explore given recent reports suggesting that vancomycin MIC elevations found in GISA strains may correlate with reduced daptomycin susceptibility. It is important to note that our studies found no relationship with \textit{agr} group or function and daptomycin susceptibility. Although addressing the most clinically relevant MRSA (USA100 \textit{agr} II and USA300 \textit{agr} I), this study was limited by not evaluating groups III and IV. The association between \textit{agr} group, function, vancomycin resistance and potential therapeutic modalities to prevent resistance in \textit{S. aureus} requires further investigation. Furthermore, the availability of several therapeutic agents to treat MRSA obligates further study to determine specific niches for these drugs with respect to variables such as vancomycin susceptibility, host factors and site of infection.

Transparency declarations

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References