Determining accurate vancomycin MIC values for methicillin-resistant *Staphylococcus aureus* by the microdilution method

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Received 21 May 2013; returned 24 June 2013; revised 3 July 2013; accepted 7 July 2013

**Objectives**: To model the standard broth microdilution method, based on a modified Gompertz function, to obtain accurate vancomycin MIC values for methicillin-resistant *Staphylococcus aureus* (MRSA). The effect of these MIC values on the vancomycin therapeutic target of $\text{AUC}_{0-24}/\text{MIC} \geq 400$ was evaluated.

**Methods**: Three clinical isolates of MRSA with different vancomycin MIC values were used in this model. The optical densities (OD) of each MIC determination were modelled by a non-linear regression method using an $F$-test. The OD data were adjusted to the Gompertz equation to obtain the MIC values. The mean vancomycin $\text{AUC}_{0-24}$ obtained with a 30 mg/kg/day dosing schedule was calculated using a Monte Carlo simulation over 5000 subjects, using the pharmacokinetic data obtained in vancomycin-treated patients in our hospital.

**Results**: Although the MIC values obtained with this model were lower than those of the diffusion method (Etest) in all three cases, this did not affect the $\text{AUC}_{0-24}/\text{MIC}$ ratio for the strains with MICs of 1 mg/L by Etest. However, in those strains with MIC values $>1$ mg/L, the confidence intervals obtained for this ratio included values $<400$.

**Conclusions**: The inherent variability of the broth microdilution method could explain the differences in the clinical outcome in MRSA-infected patients treated with vancomycin, mainly in those due to strains with MIC values of 1.5–2 mg/L by Etest, because the corresponding MIC values would range from 0.84 to 1.52 mg/L by the microdilution method, which could affect the therapeutic target.

**Keywords**: pharmacokinetics, pharmacodynamics, susceptibility testing

**Introduction**

The efficacy of vancomycin treatment in patients with methicillin-resistant *Staphylococcus aureus* (MRSA) infection is associated with an area under the concentration–time curve to MIC ratio [$\text{AUC}_{0-24}$ (mg·h/L)/MIC (mg/L)] of $\geq 400$. In patients infected with MRSA strains with vancomycin MIC values $>1$ mg/L, poor clinical results are obtained after vancomycin standard dosage therapy. In addition, the bacteraemia duration and recurrence, hospitalization and mortality rates are directly proportional to vancomycin MIC values. In these clinical trials, the correlation with poor outcomes is primarily based on MIC values determined by the agar diffusion method with antibiotic concentration gradient strips (Etest; bioMérieux, Marcy-l’Étoile, France), which is not the reference method, although it is approved by the American Society for Microbiology and European Society for Clinical Microbiology and Infectious Diseases.

Therefore, to achieve an optimal value of $\text{AUC}_{0-24}/\text{MIC} \geq 400$ is relevant to predicting good clinical outcomes in MRSA infections and changes in the vancomycin MIC value due to the method used for its determination could also be very relevant.

The standard microdilution method involves a semi-quantitative test, which gives an approximation of the lowest concentration of antibiotic required to prevent microbial visible growth. The range of antibiotic concentrations used for MIC determination involves log$_{2}$ vancomycin dilutions from 8 to 0.016 mg/L.

The standard microdilution method has a recognized accuracy of one dilution interval; therefore, for MIC values of 1 mg/L, this method accepts variations between 0.5 and 2 mg/L. Thereby, the differences found in the vancomycin MIC values obtained by Etest and microdilution can be due to the inherent variability of the latter, so it would be highly desirable to develop a method to achieve a more accurate determination of MIC values by the microdilution method.

Lambert and Pearson described a non-linear regression model for the MIC determination of disinfectants using values obtained by the microdilution method. The MIC, in that model, is defined mathematically based on a Gompertz function modified to fit the data of...
the reference method \((\text{MIC} = 10^{(M + 1/B)})\), where \(B\) is a slope parameter and \(M\) is the log concentration of the inflection point.

The aim of this study was to adapt Lambert and Pearson’s mathematical model\(^5\) to obtain more accurate vancomycin MIC values for MRSA isolates by the microdilution method and to evaluate its influence on the pharmacodynamic therapeutic target of \(\text{AUC}_{0-24}/\text{MIC} \geq 400\).

Methods

Three MRSA clinical isolates (119049, 119731 and 119000) with vancomycin MICs of 1, 1.5 and 2 mg/L, respectively, as determined by Etest (bioMérieux, France), were used to determine the vancomycin MIC by the broth microdilution method, with log\(_2\) dilutions ranging from 8 to 0.016 mg/L, according to CLSI recommendations,\(^6\) and a second MIC determination using log\(_2\) dilutions ranging from 6 to 0.02 mg/L to mimic the concentrations used by the Etest method.

The microdilution plates were incubated at 37°C in air for 24 h and then the optical density of each well, at 600 nm (OD\(_{600}\)), was determined with a spectrometer plate reader (UVM-340; ASYS Hitech, Austria). The MIC was determined in triplicate for each bacterial isolate and \(S.\) aureus ATCC 29213 was used as quality control strain.

The OD\(_{600}\) values were modelled using a modified non-linear regression method based on an \(F\)-test (GraphPad Prism 5.0).\(^7\) OD\(_{600}\) data (bacterial growth) were adjusted by the Gompertz equation to calculate the MIC and confidence intervals (CIs) directly, and were not based on the inflection point.

The mean vancomycin AUC\(_{0-24}\) obtained for a 30 mg/kg/day dosing schedule was calculated using a Monte Carlo simulation over 5000 subjects through an application built in Microsoft Excel, in which the AUC\(_{0-24}\) (mg.h/L) was calculated as dailydose/total clearance (D\(_{24h}\)/CL). The vancomycin pharmacokinetic (PK) parameters included in this model were derived from a previous study in our hospital.\(^8\) It was assumed that all the population PK parameters were distributed as a log-normal function.

Results

The modelling curves and MIC values are shown in Figure 1. The mean AUC\(_{0-24}\) derived from the Monte Carlo simulation was 441.4 \(\pm\) 149.2 mg.h/L (90% CI 437.3 – 445.6 mg.h/L).

The MIC values (mg/L) and their 90% CIs as well as the pharmacodynamic parameter (PD) AUC\(_{0-24}/\text{MIC}\) for each strain, clinical and control, are shown in Table 1.

Discussion

Our results show that vancomycin MIC values obtained by the broth microdilution standard method are lower than those obtained by Etest, as previously described when these two techniques were compared.\(^9,10\) This fact does not seem to affect the optimal therapeutic ratio of \(\text{AUC}_{0-24}/\text{MIC} \geq 400\) in strains with MICs of \(\leq 1\) mg/L by Etest. However, in those strains where MIC values are \(> 1\) mg/L, this PD ratio could not be obtained due to the CIs found for \(\text{AUC}_{0-24}/\text{MIC}\) including values <400.

Although a low number of isolates was used, this model considered the inherent variability of the microdilution method for MIC determinations, which could explain the differences found in clinical outcomes after vancomycin treatment of MRSA infections due to strains with Etest vancomycin MIC values of 1.5–2 mg/L. The real microdilution MIC values and the CIs for these strains

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**Figure 1.** Modelling curves and vancomycin MIC values of the isolates tested (vertical dotted line corresponds to the best-fit MIC value).
would range between 0.84 and 1.52 mg/L (AUC$_{0–24}$/MIC 289.6–522.4) and consequently the optimal therapeutic ratio of AUC$_{0–24}$/MIC $\geq$ 400 would not be always obtained, affecting the response correlation in isolates that appear non-susceptible by Etest PD criteria but may in fact be susceptible or non-susceptible based on the microdilution MIC PD parameter.

The low correlation between the two susceptibility testing methods for prediction of the therapeutic response in these vancomycin-treated infections$^{11}$ can be explained by these data.

Finally, this method provides a mathematical approach to determine the exact value of the MIC by the standard microdilution method that allows a more accurate adjustment of the PK/PD parameters for vancomycin treatment of severe MRSA infections. However, randomized clinical trials are needed to elucidate the clinical utility of this new methodology for MIC determinations.

### Funding
Supported by Plan Nacional de I+D+i 2008-2011 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía y Competitividad, Spanish Network for Research in Infectious Diseases (REIPI RD12/0015)—co-financed by European Development Regional Fund “A way to achieve Europe” ERDF.

### Transparency declarations
J. P. has participated as a speaker for Wyeth, Schering-Plough, MSD, Janssen, Novartis, and Pfizer and has received research grants from Novartis and Astra-Zeneca. All other authors: none to declare.

### References
7. GraphPad Software. Fitting Bacterial Growth Data to Determine the MIC and NIC. http://www.graphpad.com/support/faqid/1365/ (3 April 2013, date last accessed).

### Table 1. Vancomycin MIC and AUC$_{0–24}$/MIC values of the isolates tested

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (mg/L)</th>
<th>Etest microdilution (90% CI)</th>
<th>AUC$_{0–24}$/MIC (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>119049</td>
<td>1</td>
<td>0.67 (0.58–0.77)</td>
<td>658.8 (754–572.9)</td>
</tr>
<tr>
<td>119731</td>
<td>1.5</td>
<td>0.99 (0.84–1.15)</td>
<td>445.9 (522.4–385.2)</td>
</tr>
<tr>
<td>119000</td>
<td>2</td>
<td>1.23 (1.06–1.52)</td>
<td>358.9 (414.5–289.6)</td>
</tr>
<tr>
<td>ATCC 29213</td>
<td>1</td>
<td>0.56 (0.52–0.59)</td>
<td>788.2 (845.1–750.8)</td>
</tr>
</tbody>
</table>