Methicillin-resistant Staphylococcus aureus vancomycin susceptibility testing: methodology correlations, temporal trends and clonal patterns

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Objectives: To determine the correlation between various vancomycin MIC testing methodologies and explore the phenomenon of MIC creep.

Methods: A total of 417 consecutive non-duplicate methicillin-resistant Staphylococcus aureus (MRSA) bloodstream isolates from Liverpool Hospital between 1997 and 2008 were retrieved. All isolates were classified using PFGE and underwent susceptibility testing for vancomycin using a standard Etest (AB bioMérieux, Solna, Sweden), Vitek2® (AST-P612; bioMérieux, Inc., Durham, NC, USA) and broth microdilution (BMD) performed as per the CLSI method.

Results: Over the 12 years, 78% (n = 326) of the isolates were multiresistant MRSA (ST239-like by PFGE, where ST stands for sequence type). The correlation between MIC testing methods was moderate with Spearman's correlation coefficients of 0.50 for BMD versus Etest (P < 0.001), 0.33 for BMD versus Vitek2® (P < 0.001) and 0.42 for Etest versus Vitek2® (P < 0.001). In general, Etest results were 1 dilution higher while the Vitek2® results were 1 dilution lower than the BMD MIC result. MIC creep was dependent on the MIC testing method and the measurement used for analysis (geometric mean MIC versus modal MIC versus frequency analysis), with creep detected for Etest regression analysis only. In contrast, the proportion of isolates with a BMD MIC ≥ 2 mg/L decreased from 16% to 9% in the latter half of the study. Modal MIC was stable over the 12 years at 1 mg/L irrespective of MIC method used.

Conclusions: Correlation between vancomycin MIC methodologies remains suboptimal. Temporal MIC trends should be interpreted with caution as these are dependent on the testing methodology and the measurement used for analysis.

Keywords: antimicrobial resistance, MIC creep, Etest, broth microdilution, Vitek2

Introduction

An MIC is a measure of antibiotic activity against bacteria such as methicillin-resistant Staphylococcus aureus (MRSA). The value obtained is interpreted against established breakpoints to assist with antibiotic treatment decisions. In the treatment of MRSA bacteremia, vancomycin MIC is important, as higher mortality has been associated with susceptible but high MICs (>1.5 mg/L by Etest). The gold standard for measuring MIC remains broth microdilution (BMD). As this test is labour intensive, laboratories generally rely on automated systems for susceptibility profiles. However, the correlation between methods remains poorly studied with most data analysing agreement based on an assumed measurement error of ±1 dilution. The role of vancomycin is to be questioned both in the treatment of an individual patient and in its use as empirical therapy as a consequence of documented shifts in vancomycin MIC against MRSA over time or MIC creep. However, this phenomenon is controversial, as it has largely been limited to single-centre studies. In the absence of MRSA typing, clonal dissemination cannot be excluded for some of these findings.
We therefore undertook this study to determine the correlation between the commonly performed MIC methods (against the gold standard BMD) and the concept of MIC creep in a fully characterized isolate collection.

**Methods**

All MRSA bloodstream infection (BSI) isolates from the initial positive blood culture from Liverpool Hospital (Sydney, Australia) were included in the study. Isolates from 1997 to 2008 were retrieved from storage (–80°C) and sub-cultured twice for 48 h on horse blood agar (HBA) prior to testing. All testing occurred over a 6 month period with no evidence of creep in MIC for the appropriate control strain (ATCC 29213) throughout the period. Results were read by two independent scientists, with discordant results between scientists resolved by a third scientist. To assess reproducibility, given the assumed error of testing (±1 dilution), a proportion of discordant results were repeated.

The MIC of vancomycin was determined by Vitek2 (Gram-positive susceptibility card; AST-P612; bioMérieux, Inc., Durham, NC, USA) and Etest (0.016–256 mg/L; AB bioMérieux, Solna, Sweden) according to the manufacturer’s instructions. In addition, all isolates underwent vancomycin susceptibility testing by BMD according to CLSI methodology with the addition of a 1.5 mg/L step. In brief, isolate suspensions prepared in Mueller–Hinton II broth (MH II; cation-adjusted; BBL, Becton Dickinson, Cockeysville, MD, USA) at a starting concentration of 1 × 10⁶ cfu/mL were incubated with increasing concentrations of vancomycin (0.0625–16 mg/L) following 24 h of incubation at 35°C in air, the MIC was recorded as the first well with complete inhibition of growth by the naked eye.

All isolates were typed using PFGE following SmaI restriction digestion and banding patterns were compared with those of characterized control isolates (supplied by G. Coombs, Royal Perth Hospital, Perth, Western Australia). In addition to PFGE banding, an isolate was only considered as ST239 (where ST stands for sequence type) if the coagulase PCR-RFLP was consistent, and the antibiogram was multiresistant (Vitek2 AST-P612).

**Analysis**

SAS version 9.2 for Windows (SAS Institute, Cary, NC, USA) was used for statistical analysis. Correlation analysis was performed on matched MIC categories with Etest results rounded up to the nearest dilution common to both methods. Linear regression analysis for temporal trends was based on geometric mean MIC measurements. For modal analysis and frequency analysis the data were split into two time periods (1997–2003 and 2004–08) to give an approximately equal number of episodes in each period.

**Results**

A total of 417 MRSA BSI isolates were identified over the 12 year period. PFGE revealed that 78.2% (n = 326) of isolates were ST239-like, with the remaining isolates non-multiresistant MRSA (nmrMRSA) clones; ST22-like (n = 39), ST1-like (n = 20), ST30-like (n = 11) and ST93-like (n = 9), with 12 isolates unable to be classified by PFGE (Table 1). No clonal replacement was evident during the 12 years, with community clones adding to the overall MRSA burden.

Pulsotype diversity within ST239 was large, with 60 different clusters with Etest results rounded up to the nearest dilution common to both methods. Linear regression analysis for temporal trends was based on geometric mean MIC measurements. For modal analysis and frequency analysis the data were split into two time periods (1997–2003 and 2004–08) to give an approximately equal number of episodes in each period.

**Table 1.** Distribution of MRSA clones by time period and MIC creep determination by MIC method

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<tbody>
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<td>ST239-like</td>
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<td>45</td>
<td>61</td>
<td>67</td>
<td>66</td>
<td>69</td>
<td>326</td>
</tr>
<tr>
<td>nmrMRSA-like</td>
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<td>4</td>
<td>16</td>
<td>11</td>
<td>15</td>
<td>13</td>
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**Vancomycin MIC creep**

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</thead>
<tbody>
<tr>
<td>geometric mean MIC</td>
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<td>1.06</td>
<td>1.32</td>
<td>1.02</td>
<td>1.28</td>
<td>1.08</td>
<td>NS</td>
</tr>
<tr>
<td>frequency of isolates ≥2 mg/L</td>
<td>6%</td>
<td>0%</td>
<td>32%</td>
<td>8%</td>
<td>12%</td>
<td>0%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>modal MIC</td>
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<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>NS</td>
</tr>
<tr>
<td>geometric mean MIC</td>
<td>0.96</td>
<td>0.90</td>
<td>1.26</td>
<td>1.01</td>
<td>1.02</td>
<td>1.42</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>frequency of isolates ≥2 mg/L</td>
<td>6%</td>
<td>0%</td>
<td>34%</td>
<td>10%</td>
<td>12%</td>
<td>19%</td>
<td>NS</td>
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<tr>
<td>modal MIC</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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</tr>
<tr>
<td>geometric mean MIC</td>
<td>0.68</td>
<td>0.87</td>
<td>0.97</td>
<td>0.98</td>
<td>0.79</td>
<td>0.92</td>
<td>NS</td>
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<td>7%</td>
<td>7%</td>
<td>12%</td>
<td>8%</td>
<td>9%</td>
<td>NS</td>
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<tr>
<td>modal MIC</td>
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<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>NS</td>
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</table>

aTyping based on PFGE compared with known multilocus sequence typing (MLST) clones; 326 (78%) isolates were ST239-like and the remaining isolates were nmrMRSA and resembled ST22 (n = 39), ST1 (n = 20), ST30 (n = 11) and ST93 (n = 9), with 12 isolates (data not shown in table) unable to be classified by PFGE.

bData analysis for MIC creep was limited to ST239-like isolates only (however, similar associations with nmrMRSA were detected).

cLinear regression analysis was performed for geometric mean analysis while modal and isolate frequency analysis were performed after splitting the data into two time periods (1997–2003 and 2004–08). P values are given (NS = not significant; P > 0.05).
Despite good overall agreement (95%; results are considered equivalent if they are identical or vary by $\pm 1$ dilution) with similar MIC distributions (Figure 1), the MIC$_{50}$ and MIC$_{90}$ for both BMD and Etest were 1 mg/L and 2 mg/L, while the MIC$_{50}$ and MIC$_{90}$ for Vitek$^2$ were 0.5 mg/L and 1 mg/L. The correlation between MIC methods was moderate with Spearman’s correlation coefficients of 0.50 for BMD versus Etest ($P<0.001$), 0.33 for BMD versus Vitek$^2$ ($P<0.001$) and 0.42 for Etest versus Vitek$^2$ ($P<0.001$). Etest consistently reported MICs 0.5–1 dilution higher than BMD at low MICs (1–1.5 mg/L) with concordance or 1 dilution lower at high BMD MICs (2 mg/L) (Figure 2). In contrast, Vitek$^2$ MIC results were 1–2 dilutions lower than BMD for both low and high BMD MICs. Similar results were obtained when Etest was compared with Vitek$^2$; with Vitek$^2$ MIC results were on average 1 dilution lower than Etest MIC results.

Both vancomycin-intermediate $S$. aureus (VISA) isolates (BMD MICs of 4 mg/L) were classified as susceptible by Etest (MICs of 1.5 and 3 mg/L) and Vitek$^2$ (MICs of 0.25 and 1 mg/L). Conversely, Vitek$^2$ defined one susceptible isolate (BMD MIC of 2 mg/L) as intermediate. Similarly, one isolate was incorrectly defined as intermediate (BMD MIC of 2 mg/L) by Etest. One additional isolate, if rounded up to the next dilution (i.e. MIC 3 mg/L rounded to 4 mg/L), would be misclassified by Etest.

as intermediate. Similarly, one isolate was incorrectly defined as intermediate (BMD MIC of 2 mg/L) by Etest. One additional isolate, if rounded up to the next dilution (i.e. MIC 3 mg/L rounded to 4 mg/L), would be misclassified by Etest (Figure 3).

A high level of concordance (99%) with the initial result was obtained in the proportion (49.4%; 123/249) of discordant test results that were repeated, confirming good reproducibility.

There was a significant difference between the geometric mean MIC of ST239-like clones and nmrMRSA clones, irrespective of method (1.16 mg/L versus 0.82 mg/L for nmrMRSA clones by BMD; $P<0.01$). Similar results were obtained for Etest and Vitek$^2$. MIC creep analysis was limited to ST239-like episodes (Table 1). Associations did not change if nmrMRSA clones were included in an overall analysis (data not shown). The presence of MIC creep was dependent on the method and variable used for analysis. Geometric mean MIC increased significantly over time by Etest only ($P<0.01$). In contrast, there was evidence of significant MIC decrease over time when using frequency analysis by BMD only, with the proportion of isolates with an MIC $\geq 2$ mg/L declining from 19% in time period 1 to 6% in time period 2 ($P<0.01$). However, irrespective of method, the modal MIC was stable over time at 1 mg/L.

**Discussion**

This study confirms the previously reported moderate correlation between the gold standard BMD and alternative MIC methodologies (Vitek$^2$ and Etest). Etest results were consistently higher than BMD at low MICs ($\leq 1$ mg/L). Similar to a recent study, Etest results were either concordant or 1 dilution lower than the gold standard at MIC = 2 mg/L. Vitek$^2$ was consistently 1–2 dilutions lower than BMD. Thus automated susceptibility testing would consistently underreport high MIC isolates. This is of concern given the association between MIC and outcome and would suggest non-automated MIC testing for all invasive MRSA isolates. However, the current treatment guidelines recommend treatment decisions be based on clinical
Central tendency is the modal MIC, which remained stable over data confirm previous suggestions that the best measure of been detected by all variables (data not shown). Therefore, our Vitek2 probably only feasible on a subset of MRSA isolates. Cant laboratory implications, with non-automated MIC testing methodologies remain moderate, therefore, in laboratories that use automated platforms, formal BMD MIC testing with Etest is the methodology of choice. This has significant laboratory implications, with non-automated MIC testing probably only feasible on a subset of MRSA isolates.

MIC creep was detected over the 12 years for Etest, but not for Vitek® and BMD based on geometric mean measurements only. The strengths of our study were that all isolates were typed and MIC creep analysis was limited to the predominant clone, thus eliminating the influence of clonal dissemination as a possible explanation. In addition, as testing was performed over 6 months, we were able to exclude batch-to-batch variation as a contributing factor in our results. Of note, MIC decrease was detected when analysing the proportion of isolates with an MIC ≥2 mg/L with BMD only. However, from the data it is clear that the selection and duration of the study period has a profound effect on these variables; for example, if the study interval ended in 2002, creep would have been detected by all variables (data not shown). Therefore, our data confirm previous suggestions that the best measure of central tendency is the modal MIC, which remained stable over the entire time period, irrespective of method.

Possible explanations for the large variation in the high-MIC isolates remain unclear, and warrant further study. However, our data suggest that evolution of high-MIC isolates requires a high MRSA burden in at-risk patients in the appropriate hospital niche. This would explain the high-MIC ‘outbreak’ detected in 2001–02. Changes to subsequent rates may reflect changes in vancomycin dosing and target trough levels over time, although no changes in dosing policies were introduced over the 12 years.

A potential limitation of our study is that intra-observer variability was not corrected for over the 6 month testing period. However, this variable was reduced by undertaking two independent readings of the MIC, with discordant results resolved by a third person blinded to the other results. Similarly there was a high level of concordance when reproducibility was assessed.

In summary, vancomycin MIC creep is dependent on the method and measurement used for analysis and could not be detected when using the modal MIC. Correlations between testing methodologies remain moderate, therefore, in laboratories that use automated platforms, formal BMD MIC testing should be considered in the appropriate subset of patients.

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**Transparency declarations**

None to declare.

**References**


