Relevance of vancomycin-intermediate susceptibility and heteroresistance in methicillin-resistant Staphylococcus aureus bacteraemia

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Objectives: To assess the relevance of vancomycin-intermediate susceptibility (VISA) and heteroresistance (hVISA) in methicillin-resistant Staphylococcus aureus (MRSA) bacteraemia.

Methods: We determined vancomycin MICs for 371 saved MRSA blood isolates (2002–03; 2005–06) by Etest and broth microdilution (BMD), screened for hVISA (Etest methods), determined the population analysis profile (PAP)/AUC for isolates with suspected reduced susceptibility (MICs >2 mg/L and/or hVISA-screen-positive versus Mu3 (hVISA control), and stratified patient characteristics and outcome according to susceptibility phenotype: VISA (PAP/AUC >1.3), hVISA (PAP/AUC 0.9–1.3), and susceptible (S-MRSA; PAP/AUC <0.9).

Results: PAP/AUC revealed 6 (1.6%) VISA and 30 (8.1%) hVISA phenotypes. The Etest MIC was above the susceptibility cut-off (2 mg/L) for all VISA isolates, whereas the BMD MIC was within the susceptibility range in two (33.3%) instances. Eight hVISA isolates (26.7%) with MICs of 2 mg/L were hVISA-screen negative. SCCmec typing revealed SCCmec II in 100% of VISA, 86.7% of hVISA and 75.5% of S-MRSA isolates (P=0.04). Prior vancomycin use was documented in 100% of VISA, 73.3% of hVISA and 52.2% of S-MRSA cases (P=0.002). Outcome (compared in 243 vancomycin-treated patients with MICs of 2 mg/L) revealed longer time to clearance in VISA cases [12.1±13.1 days versus 3.3±3.9 (hVISA) and 3.7±5.1 (S-MRSA); P=0.001], more frequent endocarditis [33.3% versus 9.1% (hVISA; P=0.1) and 4.2% (S-MRSA; P=0.001)] and attributable mortality [33.3% versus 9.1% (hVISA; P=0.1) and 8.4% (S-MRSA); P=0.08].

Conclusions: No adverse outcome was documented with hVISA phenotype, whereas VISA contributed to vancomycin treatment failure. VISA and hVISA appear to emerge in SCCmec II isolates among vancomycin-exposed patients and are better detected by Etest.

Keywords: VISA, hVISA, outcome, MRSA

Introduction

The outcome of vancomycin treatment in infections with methicillin-resistant Staphylococcus aureus (MRSA) appears to be influenced by vancomycin MIC.1–7 Due to the reported high failure rate in isolates with higher MIC, the susceptibility cut-off was lowered and higher vancomycin trough levels were advocated in recent treatment guidelines.8,9 The recommendations were based on case reports, retrospective studies of a small number of vancomycin intermediate susceptible S. aureus (VISA) cases, and a survey of the Emerging Infectious Diseases Network.8,10,11 The guidelines and cut-off ranges are based on studies that used the broth microdilution (BMD) or agar dilution method.8 This interpretation of MIC–outcome association, however, may be imprecise because of the known variability of all MIC testing methods and the acceptable ± one 2-fold dilution variation.12,13 Based on these variations, isolates with vancomycin MICs at the cut-off for susceptible and intermediate susceptible classifications (2 and 4 mg/L) may be interpreted differently upon retesting. In addition to VISA, isolates with heteroresistance (hVISA) are increasingly reported.14–24 Their relevance, however, has not been substantiated due to variation in testing methods, the wide range of reported frequency, the association with vancomycin MIC at the higher end of the susceptibility range and possible selection bias among cases with less favourable outcome.8,11

During testing of our MRSA blood isolates saved from two previous observational studies,25,26 we identified isolates with

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reduced vancomycin susceptibility (MICs >2 mg/L) by Etest or BMD that were not detected by the automated VITEK 1 or 2 methods used in our clinical laboratory. We also found several isolates that screened positive for hVISA using Etest screening methods.1 We determined the population analysis profile (PAP) of all isolates with suspected reduced susceptibility to confirm their susceptibility phenotype, and compared their outcome with that of patients infected with more susceptible isolates.

Methods

Isolates

One isolate from each patient was selected from all MRSA blood isolates saved in our laboratory during two prior prospective S. aureus bacteraemia studies in 2002–03 and 2005–06.5,26 We chose the last available blood culture isolate from patients with multiple positive cultures to maximize the probability of encountering reduced susceptibility. Isolates were identified as MRSA according to standard methods and preserved in skimmed milk at −80°C until testing.

SCCmec typing was previously performed for all MRSA isolates by multilocus PCR using 18 primers (Applied Biosystems, Foster City, CA, USA) targeting SCCmec types I, II, III, Iva, Ivb, Ivc, Iv d and V and the mecA gene (internal control)1 according to Zhang et al.27

MIC testing methods

BMD

Vancomycin (Sigma-Aldrich, St Louis, MO, USA) stock suspension of 10000 mg/L was diluted to 64 mg/mL in Mueller–Hinton broth (Oxoid; Remel, Lenexa, KS, USA). Serial 2-fold dilutions (32–0.06 mg/mL) were prepared and isolates were tested in duplicate according to standard procedures and in parallel with Etest MIC.

Etest

The Etest (bioMérieux) was performed according to the manufacturer’s instructions. Results were rounded up to the nearest vancomycin concentration.

Screening for hVISA

All isolates were previously screened for hVISA phenotype by the Etest macromethod (MAC; AB Biodisk) and glycopeptide resistance detection (GRD) according to the manufacturer’s instructions.1

PAP/AUC

PAP was determined according to the Wootton et al.28 method with modification for all isolates that had vancomycin MICs >2 mg/L and/or were hVISA-screen-positive by either testing method, 11 randomly selected additional isolates with concordant hVISA-screen-negative results plus MICs <2 mg/L and all consecutive 2002–03 isolates. Briefly, serial 10-fold dilutions of organism were inoculated onto increasing concentrations of vancomycin brain heart infusion (BHI) agar (Hardy Diagnostics, Santa Maria, CA, USA). Colony growth at 48 h was counted and graphed as log_{10} CFU/mL versus vancomycin concentration (0, 1, 2, 3, 4 and 6 mg/L). ATCC control strains 29213 (methicillin-susceptible S. aureus), 700699 (Mu50; VISA) and 700698 (Mu3; hVISA) were included with each run. PAP/AUC was measured for each sample and the ratio of test isolate AUC/mean Mu3 AUC was calculated.29

Definitions

VISA was defined as PAP/AUC ratio >1.3; hVISA was defined as PAP/AUC ratio 0.90–1.3; susceptible MRSA (S-MRSA) was defined as PAP/AUC ratio <0.90. All isolates with MICs ≤2 mg/L and concordant negative hVISA screens were presumed to be fully susceptible.

Clinical data

The following patient information was collected: demographics, underlying conditions, modified Charlson co-morbidity score,26,29 source of infection, type of therapy, vancomycin trough concentrations, and treatment outcomes, including status at hospital discharge, duration of bacteremia (days between first and last positive blood culture), and metastatic foci. All hVISA-infected patients were compared with those infected with VISA and S-MRSA.

The study was conducted in a 600-bed teaching hospital in the Detroit metropolitan area. It was approved by the St John Hospital and Medical Center Institutional Review Board.

Statistical methods

Associations between susceptibility status and categorical variables were analysed with the χ² test. Student’s t-test was used to compare mean co-morbidity score and duration of bacteraemia. All statistical tests were performed using the computer software SPSS release 12. A P value <0.05 was considered to indicate statistical significance.

Results

We tested 371 saved MRSA blood isolates. The majority (n=289; 77.9%) were collected on the first day of bacteraemia; the remainder were from days 2–31 (median 6 days) of bacteraemia. Vancomycin MIC >2 mg/L was noted for 27 isolates (7.3%); it was noted by both BMD and Etest in 14 (3.8%), Etest only in 11 (3.0%) and BMD only in 2 (0.5%) instances. Screening for hVISA was positive in 76 (20.5%) isolates. PAP was determined in 221 isolates, including all isolates with suspected reduced susceptibility (Figure 1). All other isolates that had vancomycin MICs ≤2 mg/L by Etest and BMD and were also hVISA-screen negative by MAC and GRD were considered S-MRSA. PAP/AUC ratios were stratified according to MIC value (Figure 2). PAP revealed six (1.6%) VISA and 30 (8.1%) hVISA isolates. All isolates with a PAP/AUC ratio consistent with VISA were identified by Etest with MICs >2 mg/L susceptibility cut-off (3 mg/L in five cases and 4 mg/L in one case), whereas two (3.3%) were classified as susceptible (MICs of 2 mg/L) by BMD. A summary of our VISA cases is presented in Table 1.

The relevance of VISA and hVISA was assessed among isolates with vancomycin MICs ≥2 mg/L. All patients infected with a more susceptible isolate (≤1.5 mg/L) were excluded. Comparing patients according to their susceptibility phenotype showed similar characteristics except for a trend towards higher haemodialysis dependence among VISA and hVISA cases and higher frequency of diabetes and having an endovascular source among VISA cases (Table 2). We did not keep track of vancomycin exposure in all patients, but a history of prior vancomycin treatment was documented in all VISA and most hVISA cases. All VISA and most hVISA isolates were SCCmec type II.

Treatment outcome was compared among 243 patients treated with vancomycin for ≥2 days, including all VISA and 22 hVISA cases. The remainder were excluded because they did not
receive effective anti-MRSA therapy (n = 14), they received vancomycin for 1 day only (n = 11), care was withdrawn (n = 7) or they received alternative therapy (n = 6). No difference was noted in the presence of a removable source, time to source removal, time to first dose of antibiotics and the average vancomycin trough level. No significant difference was noted in any outcome measure between hVISA and S-MRSA cases (Figure 3). In comparison, VISA cases had a higher incidence of endocarditis (P = 0.001 versus S-MRSA) and tended to have higher attributable mortality (P = 0.08 versus S-MRSA). The differences between VISA and hVISA cases were not significant (Table 2). Additionally, the duration of bacteremia among VISA cases was significantly longer compared with hVISA and S-MRSA cases, whereas no difference was noted between hVISA and S-MRSA cases (Figure 4).

**Discussion**

The relevance of MRSA isolates with reduced vancomycin susceptibility remains poorly documented due to non-standardized testing methods for hVISA and few reported cases of VISA. VISA is defined as isolates with MICs of 4–8 mg/L. Classifying the organisms based on MIC results, however, may have some
limitations due to the acceptable ± one 2-fold dilution variations in all testing methods. PAP is probably more precise, but not used in the definition. In this study, we determined the PAP for all isolates with suspected reduced susceptibility and stratified cases according to their PAP/AUC ratio.

Our findings show that VISA and hVISA phenotypes account for a minority of MRSA bacteraemia and are encountered among patients with prior vancomycin exposure, although we did not have access to all medical encounters for the remaining patients, which limits accurate determination of prior vancomycin exposure.

Table 1. Summary of patients with VISA bacteraemia

<table>
<thead>
<tr>
<th>Case</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60</td>
<td>57</td>
<td>37</td>
<td>54</td>
<td>74</td>
<td>47</td>
</tr>
<tr>
<td>Sex</td>
<td>male</td>
<td>male</td>
<td>female</td>
<td>male</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td>Prior vancomycin therapy</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Source</td>
<td>IVC</td>
<td>IVC</td>
<td>IVC-IE</td>
<td>AVG</td>
<td>UK</td>
<td>PMV-IE</td>
</tr>
<tr>
<td>Removable focus (day of removal)</td>
<td>yes (9)</td>
<td>yes (0)</td>
<td>no</td>
<td>yes (0)</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Echocardiogram</td>
<td>ND</td>
<td>ND</td>
<td>TEE (+)</td>
<td>TTE (-)</td>
<td>TEE (-)</td>
<td>TEE (+)</td>
</tr>
<tr>
<td>Etest MIC (mg/L)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>BMD MIC (mg/L)</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>PAP/AUC ratio</td>
<td>1.34</td>
<td>1.32</td>
<td>1.32</td>
<td>1.45</td>
<td>1.57</td>
<td>1.41</td>
</tr>
<tr>
<td>Vancomycin trough</td>
<td>12.2</td>
<td>ND</td>
<td>12.7</td>
<td>20.4</td>
<td>15.3</td>
<td>33.9</td>
</tr>
<tr>
<td>Duration of bacteraemia (days)</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>15</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Outcome</td>
<td>expired</td>
<td>expired</td>
<td>relapse</td>
<td>UK</td>
<td>cured</td>
<td>expired</td>
</tr>
</tbody>
</table>

IVC, intravenous catheter; IE, infective endocarditis; AVG, dialysis arteriovenous graft; UK, unknown; PMV, prosthetic mitral valve; ND, not done; TTE, trans-thoracic; TEE, trans-oesophageal.

Table 2. Comparative characteristics and outcome stratified according to susceptibility

<table>
<thead>
<tr>
<th>Susceptibility phenotype (no. of cases)</th>
<th>VISA (6)</th>
<th>hVISA (30)</th>
<th>S-MRSA (245)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ageb (years)</td>
<td>54.8 ± 12.5</td>
<td>59.8 ± 14.2</td>
<td>63.0 ± 16.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Co-morbidityc scoreb</td>
<td>4.8 ± 2.0</td>
<td>3.8 ± 1.8</td>
<td>3.3 ± 1.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Vancomycin exposure</td>
<td>6 (100)</td>
<td>22 (73.3)</td>
<td>128 (52.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>Haemodialysis</td>
<td>2 (33.3)</td>
<td>9 (30.0)</td>
<td>48 (19.6)</td>
<td>0.3</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (66.7)</td>
<td>14 (46.7)</td>
<td>93 (38.0)</td>
<td>0.3</td>
</tr>
<tr>
<td>Vascular source</td>
<td>5 (83.3)</td>
<td>14 (46.7)</td>
<td>98 (40.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>Removable focus</td>
<td>3 (50.0)</td>
<td>10 (33.3)</td>
<td>86 (35.1)</td>
<td>0.3</td>
</tr>
<tr>
<td>Days to removalb</td>
<td>2.2 ± 3.9</td>
<td>4.9 ± 6.3</td>
<td>3.2 ± 5.3</td>
<td>0.6</td>
</tr>
<tr>
<td>SCCmec type II</td>
<td>6 (100)</td>
<td>26 (86.7)</td>
<td>185 (75.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>Treatment with vancomycin</td>
<td>6 (100)</td>
<td>22 (73.3)</td>
<td>215 (87.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>Time to treatmentb (h)</td>
<td>22.6 ± 21.9</td>
<td>27.5 ± 27.2</td>
<td>23.3 ± 27.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Vancomycin trough</td>
<td>18.9 ± 10.2 (6)</td>
<td>13.4 ± 6.4 (21)</td>
<td>14.1 ± 6.2 (188)</td>
<td>0.2</td>
</tr>
<tr>
<td>Trough ≥ 10 mg/L</td>
<td>5 (83.3)</td>
<td>16 (76.2)</td>
<td>138 (73.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>Outcomesb (no. of cases)</td>
<td>(6)</td>
<td>(22)</td>
<td>(215)</td>
<td></td>
</tr>
<tr>
<td>Duration of bacteraemia</td>
<td>12.1 ± 13.1</td>
<td>3.3 ± 3.9</td>
<td>3.7 ± 5.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>2 (33.3)</td>
<td>2 (9.1)</td>
<td>9 (4.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>3 (50.0)</td>
<td>5 (22.7)</td>
<td>60 (27.9)</td>
<td>0.4</td>
</tr>
<tr>
<td>Attributable mortality</td>
<td>2 (33.3)</td>
<td>2 (9.1)</td>
<td>18 (8.4)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

aResults are presented as n (%), unless otherwise indicated.
bMean ± SD.
cCharlson’s co-morbidity score.
dmg/L.
eDetermined among vancomycin-treated patients.
fDuration in days.

Table 1. Summary of patients with VISA bacteraemia

Table 2. Comparative characteristics and outcome stratified according to susceptibility
Our results illustrate that vancomycin treatment in patients with VISA bacteremia was associated with significant delay in clearance and a trend towards higher attributable mortality. No such adverse outcome was noted with the hVISA phenotype when compared with S-MRSA. This confirms our previous observation of the lack of relevance of hVISA, which was based on screening methods.1

The relevance of hVISA, described in detail by Tenover and Moellering,11 remains controversial. One of the possible reasons for this uncertainty is the strong association between hVISA phenotype and MIC at the high end of the susceptibility range, limiting the distinction between the role of hVISA and high MIC.3,4,19 Although the hVISA phenotype was described in isolates with MICs as low as 0.5 mg/L, their frequency was too low for reliable conclusions.29–31 Additionally, the use of diverse screening methods adds to the uncertain relevance of hVISA.9 Other possible reasons may include the poor distinction between S-MRSA and hVISA or hVISA and VISA, since reduced susceptibility represents a continuum with artificial cut-offs.32

Possible limitations of our study include the method for isolate selection, which may favour the detection of reduced susceptibility among patients with persistent bacteremia. Whether reduced susceptibility contributes to worse outcome or whether treatment failure and persistent bacteremia provide the milieu for the emergence of hVISA and VISA is uncertain. Five (83.3%) of the VISA phenotypes were noted in isolates recovered on days 5–33 of bacteremia, whereas most S-MRSA (78.6%) and hVISA (77.3%) phenotypes were detected on the first day of bacteremia.

In summary, we present the largest series of well-confirmed VISA bacteremia from a single medical centre and stratify the outcome based on the susceptibility phenotype validated by population analysis profile in all isolates with suspected reduced susceptibility. We selected patients with isolates at the upper limit of the susceptibility range for better assessment of hVISA relevance. Our findings illustrate that the outcome of hVISA bacteremia is similar to that of fully susceptible isolates, suggesting that the hVISA phenotype is not clinically relevant, and substantiate that vancomycin treatment in VISA cases is associated with a high failure rate.

The relevance of hVISA and its accurate epidemiology will remain controversial until a reliable and practical testing method becomes standardized and adopted for use in clinical laboratories and future studies.

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**Transparency declarations**
L. B. J. served as a consultant for Cerexa, Inc. All other authors: none to declare.

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