Occurrence of vancomycin-tolerant and heterogeneous vancomycin-intermediate strains (hVISA) among Staphylococcus aureus causing bloodstream infections in nine USA hospitals

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Background: The bactericidal activities of vancomycin and daptomycin were evaluated in a large collection of methicillin-resistant Staphylococcus aureus (MRSA) bacteraemia strains from nine major USA medical centres.

Objectives: To evaluate the occurrence of heterogeneous vancomycin-intermediate S. aureus (hVISA) among MRSA strains tolerant to vancomycin and/or with increased vancomycin or daptomycin MIC values. The accuracy of the macro Etest method (MET) compared with population analysis profiling (PAP) for the detection of hVISA was also assessed.

Methods: A total of 1800 MRSA strains were collected from bloodstream infections at the nine sites (40 strains per year, per medical centre during the 2002–06 study period). Vancomycin and daptomycin MIC testing was performed by reference broth microdilution (all strains) and MBC tests on 50% of strains (randomly selected). A subset of isolates (n=268) having an increased vancomycin MBC (≥16 mg/L), an increased vancomycin MIC (≥1 mg/L) and/or an increased daptomycin MIC (>0.5 mg/L) were tested for susceptibility to vancomycin and teicoplanin by MET.

Results: Overall, 181 of 900 (20.1%) MRSA tested exhibited vancomycin tolerance, varying from 10% to 43% among the medical centres evaluated, and from 11.7% in 2004 to 27.8% in 2005. No resistance trend was observed in any medical centre or in the overall study data. Daptomycin showed bactericidal activity against all strains tested. The accuracy of MET for identifying hVISA strains varied significantly with the criteria applied for positivity.

Conclusions: The most frequently used criteria to define hVISA, i.e. MET reading values ≥8 mg/L for both vancomycin and teicoplanin or ≥12 mg/L for teicoplanin only, detected 20 of 36 PAP-positive strains (55.6% sensitivity), indicating that the prevalence of hVISA could be higher than currently appreciated. Daptomycin was bactericidal against hVISA strains.

Keywords: daptomycin, MRSA, vancomycin tolerance, macro Etest method, teicoplanin

Introduction

An unfavourable clinical response to vancomycin treatment associated with vancomycin-tolerant or heterogeneous vancomycin-intermediate Staphylococcus aureus (hVISA) strains has been increasingly reported.¹⁻⁵ Although the superiority of bactericidal over bacteriostatic antimicrobials is very controversial and may not be translated to better clinical outcome in the majority of infections, there are those infection types that may require effective bactericidal action, such as endocarditis, meningitis, osteomyelitis and systemic infections in immunocompromised patients.⁶⁻⁹ hVISA are strains of vancomycin-susceptible S. aureus (MIC, ≤2 mg/L) containing subpopulations of vancomycin-intermediate cells. hVISA may represent the stage that precedes the development of intermediate-level vancomycin resistance in S. aureus (VISA). Continued exposure to vancomycin would favour the outgrowth of this subpopulation leading to a uniform VISA population.¹⁰ Since the recognition of hVISA, it has been suggested that the presence of this vancomycin-intermediate subpopulation is responsible for some unfavourable clinical outcomes.²⁻⁴,⁵ However, the difficulty involved in the identification of hVISA strains complicates any routine determination of their clinical significance and role in treatment failure.

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hVISA causing bacteraemia in USA hospitals

There are no standard methods or clear international guidelines for detection of hVISA. Population analysis profiling (PAP) is considered the ‘gold standard’, but this approach is labour intensive and costly.\textsuperscript{11,12} The macro Etest method (MET; AB BIODISK, Solna, Sweden) exhibited high sensitivity and specificity compared with PAP in initial studies, and has been used for screening or as the definitive test to assess the prevalence of hVISA.\textsuperscript{11,13,14} The criteria most frequently used to characterize hVISA have been those described by Wootton\textit{et al.},\textsuperscript{12} e.g. MET reading values $\geq 8$ mg/L for both vancomycin and teicoplanin, or $\geq 12$ mg/L for teicoplanin alone after 48 h of incubation in brain heart infusion (BHI) agar at 35$^\circ$C.

In this study, we evaluated the bactericidal activities of vancomycin and daptomycin against a large collection of methicillin-resistant \textit{S. aureus} (MRSA) strains from nine major USA medical centres, each representing a different USA census region. We also evaluated the accuracy of MET by comparing the results of that method using various interpretive criteria with the results obtained by the modified PAP method.

Materials and methods

\textbf{Bacterial isolates and susceptibility testing}

As part of a large multicentre study, 1800 MRSA isolates from bacteraemia were collected in nine USA medical centres (one from each census region). Each medical centre was requested to randomly select 40 strains per year during the 2002–06 period (total of 200 MRSA strains per centre). All isolates were tested for susceptibility to vancomycin and daptomycin by reference broth microdilution methods according to CLSI M07-A8.\textsuperscript{15} Mueller–Hinton broth adjusted to contain physiological levels of calcium (50 mg/L) was used when testing daptomycin. MBC tests were performed for vancomycin and daptomycin on 50% of isolates (random selection) by plating the broth from the MIC endpoint well and from those four $\log_2$ dilutions above the MIC for each organism onto appropriate growth medium. Vancomycin and daptomycin tolerance was defined as an MBC/MIC ratio $\geq 32$.

\textbf{MET}

A subset of isolates ($n=268$) showing an increased vancomycin MBC ($\geq 16$ mg/L, which is the vancomycin MIC resistance breakpoint established by the CLSI;\textsuperscript{15} 223 isolates), an increased vancomycin MIC ($\geq 1$ mg/L; 57 isolates) and/or an increased daptomycin MIC ($\geq 0.5$ mg/L; eight isolates) were tested for susceptibility to vancomycin and teicoplanin by MET (AB BIODISK) using a high inoculum (2 McFarland) on BHI agar, as previously described.\textsuperscript{11,12} Twenty strains fulfilled more than one criterion. Briefly, 100 $\mu$L of suspension was evenly spread onto a 90 mm BHI agar plate (Difco, Detroit, MI, USA) and allowed to dry. Vancomycin and teicoplanin Etest strips (AB BIODISK) were applied to the surface of the agar in parallel but opposite directions, and the plates were incubated at 35$^\circ$C for 48 h. Zones were read at complete inhibition carefully observing for visual hazy growth and microcolonies.

\textbf{Modified PAP}

PAP was performed, as previously described,\textsuperscript{11,12,14} on strains with MET reading values $\geq 4$ mg/L for vancomycin and $\geq 8$ mg/L for teicoplanin, or $\geq 12$ mg/L for teicoplanin alone with MET. Briefly, organisms were cultured in supplemented Mueller–Hinton broth (SMHB) from an overnight growth, adjusted to a density of $10^8$ cfu/mL and spiral plated (Don Whitley Scientific Ltd, West Yorkshire, UK) onto BHI agar (Difco) plates containing 0, 0.5, 1, 1.5, 2, 3, 4 and 8 mg/L vancomycin. Colonies were counted 48 h after incubation at 35$^\circ$C. The resulting cfu/mL values were plotted against the vancomycin concentration. The AUC for each strain was determined by trapezoidal rule using Sigma Plot 9.0 (Richmond, CA, USA). Each strain was run in conjunction with Mu3 as the control hVISA strain (see Figure 1). A ratio was then calculated by dividing the AUC of the test strain by the AUC of Mu3. The PAP-AUC criteria for determination of hVISA have been previously described and are based on ratios of $\geq 0.90$ for hVISA and $\geq 1.3$ for \textit{VISA}.\textsuperscript{11,12} The performance of MET for detecting hVISA was compared with the PAP-AUC ratio results. Randomly selected strains (9) with low vancomycin and teicoplanin MET reading values ($< 4$ mg/L) were also evaluated by PAP as wild-type controls. Mu50 and \textit{S. aureus} ATCC 29213 were tested as additional controls and all results were within the expected ranges.

\textbf{Evaluation of the accuracy of MET for detection of hVISA strains}

Sensitivity, specificity and predictive values of various criteria for characterization of hVISA, including those published by Wootton\textit{et al.},\textsuperscript{12} were evaluated by comparing the results of MET and PAP.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of strains</th>
<th>vancomycin MBC/MIC</th>
<th>daptomycin MBC/MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>180</td>
<td>73 (40.6)</td>
<td>153 (85.0)</td>
</tr>
<tr>
<td>2003</td>
<td>180</td>
<td>73 (40.6)</td>
<td>159 (88.3)</td>
</tr>
<tr>
<td>2004</td>
<td>180</td>
<td>88 (48.9)</td>
<td>159 (88.3)</td>
</tr>
<tr>
<td>2005</td>
<td>180</td>
<td>62 (34.4)</td>
<td>166 (92.2)</td>
</tr>
<tr>
<td>2006</td>
<td>180</td>
<td>79 (43.9)</td>
<td>151 (83.9)</td>
</tr>
<tr>
<td>Total</td>
<td>900</td>
<td>375 (41.7)</td>
<td>788 (87.6)</td>
</tr>
</tbody>
</table>

Table 1. Summary of bactericidal activities of vancomycin and daptomycin by year (all medical centres)
Results

Percentages of isolates displaying vancomycin tolerance (vancomycin MBC/MIC ratios of ≥32) markedly varied by year between institutions and no clear trend in tolerance was apparent. Overall, 181 of 900 (20.1%) MRSA tested exhibited vancomycin tolerance, varying from 11.7% in 2004 to 27.8% in 2005 (Table 1). The occurrence of vancomycin-tolerant MRSA varied from 10% to 43% among the medical centres evaluated when data from all 5 years were combined, but no yearly trend was observed in any medical centre (data not shown). In contrast to the results obtained with vancomycin, daptomycin exhibited consistent bactericidal activity against the 900 strains tested (Table 1). Daptomycin MBC/MIC ratios were 1 for 788 (87.6%) and 2 for 110 (12.2%) MRSA strains. The highest daptomycin MBC/MIC ratio value was 4 [only 2 (0.2%) strains; Table 1].

Among 268 MRSA strains tested by MET, 43 exhibited a MET reading value of ≥4 mg/L for vancomycin and ≥8 mg/L for teicoplanin, or ≥12 mg/L for teicoplanin alone. These 43 strains plus 9 randomly selected strains (one per medical centre) with low vancomycin and teicoplanin MET reading values (<4 mg/L) were evaluated by PAP and 36 of those (69.2%) were characterized as hVISA by that method (Figure 1). Thus, the prevalence of hVISA among this MRSA collection containing strains tolerant to vancomycin and/or with increased vancomycin (>1 mg/L) or daptomycin MIC (>0.5 mg/L) was 13.4% (36/268). When these groups were analysed separately, isolates with an elevated vancomycin MIC (≥1 mg/L) showed the highest prevalence of hVISA strains (45.6%; 26 of 57 isolates tested); followed by isolates with an elevated daptomycin MIC (>0.5 mg/L; 25% (2 of 8)) and vancomycin-tolerant strains (9.4%; 21 of 223).

The most commonly used MET criteria for hVISA (teicoplanin ≥12 or teicoplanin ≥8 and vancomycin ≥8 mg/L) showed a sensitivity of only 55.6%, detecting only 20 of 36 PAP-positive strains (Table 2). Furthermore, three strains that met these criteria were negative by PAP, including two strains with a teicoplanin MET reading at 12 mg/L and a vancomycin MET reading at 3 mg/L. The highest sensitivity was obtained using positive MET criteria of a vancomycin MET reading at ≥4 mg/L and a teicoplanin MET reading at ≥4 mg/L (97.2%; Table 2). If a teicoplanin MET reading at ≥12 mg/L alone was included in the criteria, the specificity would decrease from 68.8% to 56.2% without any gain in sensitivity.

Discussion

S. aureus is the major cause of serious hospital- and community-acquired infections; and glycopeptides, particularly vancomycin, have been the recommended therapy for serious MRSA infections for decades. The results of this study showed that the occurrence of vancomycin-tolerant strains (MBC/MIC, ≥32) was generally elevated (20.1% overall), but without a clear trend toward increasing prevalence over time. If isolates with a vancomycin MBC≥16 mg/L, which is the vancomycin MIC resistance breakpoint established by the CLSI,15 were also considered tolerant,16 the overall occurrence of vancomycin tolerance would be 24.8% (223/900), varying from 15% to 47% among the medical centres evaluated (data not shown). In contrast to vancomycin, daptomycin was highly bactericidal against this large collection of MRSA strains from bloodstream infections (2002–06), including vancomycin-tolerant and hVISA isolates. Although the superiority of bactericidal over bacteriostatic antimicrobials remains controversial, for the treatment of high bacterial density infections such as bacterial endocarditis and serious infections in immunocompromised patients, bactericidal activity is clearly preferred.3,6–9

The first clinical isolate of S. aureus with documented decreased susceptibility to vancomycin (MIC of 8 mg/L) was reported from Japan in 1997.17 Since then, many S. aureus isolates with reduced susceptibility to glycopeptides have been reported from around the world.18 Although the clinical

Table 2. Accuracy of MET using various criteria for the detection of hVISA

<table>
<thead>
<tr>
<th>Positive MET criteria (subpopulation detected at the MIC in mg/L)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEI ≥12 or TEI ≥8 and VAN ≥8a</td>
<td>55.6</td>
<td>81.3</td>
<td>87.0</td>
<td>44.8</td>
<td>63.5</td>
</tr>
<tr>
<td>TEI ≥12 or TEI ≥8 and VAN ≥6</td>
<td>69.4</td>
<td>68.8</td>
<td>83.3</td>
<td>50.0</td>
<td>69.2</td>
</tr>
<tr>
<td>TEI ≥12 or TEI ≥8 and VAN ≥4</td>
<td>97.2</td>
<td>56.2</td>
<td>83.3</td>
<td>90.0</td>
<td>84.6</td>
</tr>
<tr>
<td>TEI ≥8 and VAN ≥4</td>
<td>97.2</td>
<td>68.8</td>
<td>87.5</td>
<td>91.7</td>
<td>88.5</td>
</tr>
</tbody>
</table>

TEI, teicoplanin; VAN, vancomycin.

aMost frequently used MET criteria to define hVISA.
significance of VISA is more well defined, and vancomycin is not recommended for treatment of infections caused by S. aureus strains with a vancomycin MIC > 2 mg/L.\textsuperscript{15} The clinical relevance of hVISA has not been completely elucidated, mainly due to the lack of standardized laboratory methods for its detection.\textsuperscript{16,17} Moreover, there is some evidence that vancomycin may not be appropriate treatment for serious infections caused by hVISA.\textsuperscript{18,19} Numerous reports of poor clinical outcome attributed to vancomycin heterogeneous resistance have been published, including a report by Moore \textit{et al.}\textsuperscript{4} that verified vancomycin treatment failure in a case of MRSA endocarditis infected with a hVISA strain. In a more recent study, Maor \textit{et al.}\textsuperscript{20} compared 27 cases with hVISA bacteremia with 223 control patients (MRSA bacteraemias) and observed that hVISA bacteremia was significantly associated with prolonged infection with greater rates of endocarditis and osteomyelitis compared with controls. Furthermore, Rose \textit{et al.}\textsuperscript{5} demonstrated that vancomycin at appropriate doses (fAUC/MIC, 105–317) had poor activity against clinical strains of hVISA in an in vitro pharmacokinetic/pharmacodynamic model. This study showed that vancomycin activity was limited against hVISA even at simulated doses of 5 g every 12 h (fAUC/MIC, 799).\textsuperscript{5}

In spite of growing evidence of the importance of heterogeneous resistance to vancomycin, there is still no standardized method or clear guidelines for detection of hVISA. MET has been widely used, and variants of this technique have been developed and applied in clinical investigations.\textsuperscript{21,22} The results of the present study indicate that the accuracy of MET and its variants, such as the Etest GRD\textsuperscript{TM} (AB BIODISK), may vary significantly with the criteria applied, thus complicating glycopeptide therapy. Of great concern is the fact that the most frequently used criteria to define hVISA\textsuperscript{15} demonstrated low sensitivity (Table 2), suggesting that the prevalence of hVISA could be higher than currently appreciated. The criteria that correlated best with PAP analysis was a teicoplanin MET reading at ≥8 mg/L and a vancomycin MET reading at ≥4 mg/L (97.2% sensitivity but a specificity of only 68.8%).

The occurrence of vancomycin-tolerant \textit{S. aureus} and hVISA strains was not uncommon among a large, geographically diverse collection of MRSA strains collected from bacteraemic patients. However, it is important to emphasize that this study was not designed to evaluate the prevalence of hVISA since the isolates tested for hVISA were pre-selected based on elevated MIC and/or MBC values of vancomycin and daptomycin.

In summary, the results of this study show that daptomycin possesses a more potent bactericidal activity compared with vancomycin and remained highly active against vancomycin-tolerant \textit{S. aureus} and hVISA strains. Furthermore, the MET criteria currently used for detection of hVISA showed compromised sensitivity and should be re-evaluated to properly detect those organisms.

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Transparency declarations

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References


