Lack of Heteroresistance among \textit{Staphylococcus aureus} Isolates with Vancomycin MICs of 2 Micrograms per Milliliter by Automated Testing

Susceptibility testing of methicillin (meticillin)-resistant \textit{Staphylococcus aureus} (MRSA) isolates from blood cultures collected at Emory University Hospital from March 2008 to July 2008 using the MicroScanWalkaway96 (software version 2.01; Siemens Healthcare Diagnostics, Inc., Deerfield, IL) identification/susceptibility system with the POS Combo29 broth microdilution panel and the PROMPT inoculation method revealed a modal vancomycin MIC of 2 \(\mu\)g/ml. All clinical MRSA blood isolates (collected between 26 March 2008 and 6 July 2008 with vancomycin MICs of 2 \(\mu\)g/ml were evaluated by additional MIC testing methods and for evidence of heteroresistant vancomycin-intermediate \textit{Staphylococcus aureus} (hVISA) isolates by the population analysis profile–area-under-the-curve (PAP-AUC) method. Thirty isolates from unique patients were identified; repeat positive MRSA cultures from the same patient were excluded. Sixty-three percent of all MRSA isolates and 56% of all methicillin-susceptible \textit{Staphylococcus aureus} isolates had vancomycin MICs of 2 \(\mu\)g/ml by MicroScanWalkaway96 during the study period.

Isolates were stored at \(-70^\circ\)C in BBL trypticase soy broth (Becton, Dickinson and Company, Sparks, MD) containing 20% glycerol and subcultured twice prior to testing. Vancomycin MICs were determined by standard Etest methods (bio-Mérieux, Durham, NC) using a 0.5 McFarland standard inoculum on Mueller-Hinton agar plates (Remel, Lenexa, KS). Reference broth microdilution vancomycin MICs were determined using standard CLSI methods (1, 2). Heteroresistance was evaluated by PAP-AUC measurement using the modified method of Wootton et al. (5), whereby \(10^{-3}\) and \(10^{-6}\) culture dilutions were spiral plated onto brain heart infusion agar containing increasing concentrations of vancomycin, and by the microdilution technique previously described by Pfeltz et al. (4). For both methods, the concentrations of vancomycin used were 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, and 8.0 \(\mu\)g/ml. Mu3 (the archetype hVISA), ATCC 29213 (vancomycin-susceptible \textit{Staphylococcus aureus}), and Mu50 (vancomycin-intermediate \textit{Staphylococcus aureus}) were included as controls (3).

The results are depicted in Table 1. All 30 isolates with initial vancomycin MICs of 2 \(\mu\)g/ml by the MicroScan method had lower vancomycin MICs by the reference broth microdilution method and Etest. The modal vancomycin MICs for all 30 isolates were 1 \(\mu\)g/ml by reference broth microdilution and 1.5 \(\mu\)g/ml by the Etest. The mean PAP-AUC ratio for the 30 MRSA clinical isolates to the hVISA strain Mu3 was 0.56, with a mode of 0.49, and no heteroresistance was detected among the 30 isolates by either method (a heteroresistant isolate was defined as one with an AUC \(\geq 0.90\) of that of Mu3).

All isolates had lower vancomycin MICs by the Etest and reference broth microdilution methods than by the automated MicroScan method; however, differences between vancomycin MICs generated by automated testing and those generated by the CLSI reference broth microdilution method of (plus or minus) a single twofold dilution are within a widely accepted margin of error. Moreover, no definitive data demonstrating significant clinical implications for a single-dilution difference in vancomycin MIC within the range of 0.5 to 2 \(\mu\)g/ml exist.

Although no hVISA isolates were identified in this collection, the emergence of MRSA with decreased vancomycin susceptibility presents major diagnostic and therapeutic challenges for clinicians and microbiologists.

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### REFERENCES


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