Validation of Vitek version 7.01 software for testing staphylococci against vancomycin☆

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Abstract

We tested 143 isolates of staphylococci with vancomycin by the National Committee for Clinical Laboratory Standards broth microdilution (BMD) reference method and compared the results to those generated using the Vitek automated system (GPS-105 and GPS-107 cards and version 7.01 software). For ten isolates, the vancomycin MICs by BMD were 8 g/ml. By Vitek, the vancomycin MICs ranged from 2 to 16 g/ml. Vancomycin MICs of ≥32 μg/ml were reported for two additional isolates by Vitek; however, the MICs decreased to ≤0.5 μg/ml on retesting. By BMD, the vancomycin MICs for both isolates were 1 μg/ml. While the modal vancomycin MIC results by BMD for S. aureus and coagulase-negative staphylococci (CoNS) were both 1 μg/ml, Vitek results showed a mode of 0.5 μg/ml for S. aureus, and a mode of 2 μg/ml for CoNS. Vitek did not report vancomycin MICs of 1 or 4 μg/ml for any of the isolates tested. While the sensitivity of detecting staphylococci with reduced susceptibility to vancomycin appears to be improved with Vitek version 7.01 software, when compared to earlier software versions, laboratories may notice an overall shift in MIC data toward higher vancomycin MICs, although for the most part, this does not affect the categorical interpretations of the results.

1. Introduction

Vancomycin remains the primary antimicrobial agent for treatment of infections caused by methicillin-resistant Staphylococcus aureus and coagulase-negative staphylococci (CoNS) (Chambers, 1997; Gilbert et al., 2001). However, there have been several recent reports of S. aureus and CoNS with reduced susceptibility to vancomycin associated with treatment failure (Garrett et al., 1999; Hiramatsu et al., 1997; Kim et al., 2000; Sieradski et al., 1999; Smith et al., 1999). In none of the cases did the vancomycin MICs exceed 16 μg/ml, which, by National Committee for Clinical Laboratory Standards (NCCLS) classification, is still considered in the intermediate range. However, concern was raised about the ability of clinical laboratories to detect this emerging resistance problem (Tenover et al. 1998).

One of the methods frequently used in laboratories to detect decreased susceptibility to vancomycin among isolates of S. aureus and CoNS is the Vitek automated system1 (bioMérieux, Hazelwood, Mo.). Earlier reports indicated that previous Vitek software versions often reported vancomycin MICs in the NCCLS susceptible range (≤0.5–4 μg/ml) for isolates that by broth microdilution (BMD) demonstrated vancomycin MICs in the intermediate range (8–16 μg/ml) (Tenover et al., 1998). Vitek recently introduced software version 7.01, which purportedly is more sensitive than earlier software versions in detecting S. aureus and CoNS with reduced susceptibility to vancomycin (Vitek Product Information, 1999). In our study, we evaluated 143 selected isolates of staphylococci to determine whether the new Vitek software improved detection of staphylococcal isolates with decreased susceptibility to vancomycin.

2. Materials and methods

2.1 Bacterial strains

143 isolates of staphylococci (Table 1) from 28 U.S. states and Japan that were submitted to the Centers for Disease Control and Prevention (CDC) or Project ICARE (Intensive Care Antimicrobial Resistance Epidemiology) (Fridkin et al., 1999; Hubert et al., 1999) for confirmation of
resistance phenotypes were selected for antimicrobial susceptibility testing. All but four *S. aureus* isolates and 16 CoNS isolates were oxacillin-resistant by BMD. The isolates were transferred from -70°C storage onto Trypticase Soy agar plates containing 5% defibrinated sheep blood (BD BioSciences, Sparks, MD) and subcultured a second time before testing. The isolates were identified by catalase and coagulase tests (Staphaurex, Murex, UK) and standard biochemical procedures (Kloos and Bannerman, 1999).

### 2.2 Study methods

The study was conducted in three phases. In Phase I, two methods, the NCCLS BMD method and the Vitek automated system, were used to determine the vancomycin susceptibility of the study isolates. For BMD, 96-well MIC plates were prepared in-house using NCCLS methods (NCCLS, 2000). Vancomycin (Eli Lilly & Co., Indianapolis, IN) was tested at concentrations of 0.06–128 μg/ml. Testing and interpretation were performed according to NCCLS document M7-A5 (NCCLS, 2000). For the Vitek method, GPS-107 cards and the version 7.01 software were used. Testing was performed following manufacturer’s instructions. In Phase II, 139 of the 143 isolates were tested using the Vitek method with both GPS-105 and GPS-107 cards; four isolates were not available for testing. In Phase III, two hospital laboratories in Atlanta (hospitals A and B) that use the Vitek system with software version 7.01 with GPS-106 cards participated in an inter-laboratory MIC comparison using a subset of isolates. Fifteen *S. aureus* strains for which the broth microdilution MICs were 1 μg/ml (seven isolates), 4 μg/ml (six), or 8 μg/ml (two) were selected. The isolates were subcultured from frozen stocks, transferred to Trypticase Soy agar plates containing 5% defibrinated sheep blood, and incubated at 35°C for 18 h. The isolates were sent to the laboratories where the isolates were subcultured a second time to blood agar plates prior to testing on the Vitek systems.

The quality control strains used for the BMD method were *Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 29213, and *S. aureus* ATCC 43300. For the Vitek method, the quality control strains used were *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, *Escherichia coli* ATCC 35218, and *E. faecalis* ATCC 51299.

### 3. Results

#### 3.1 Broth microdilution versus Vitek (Phase I)

The vancomycin MIC distributions generated by BMD and the Vitek method using the GPS-107 card are shown in Table 1. For 143 isolates of *S. aureus* and CoNS, the BMD method yielded a vancomycin MIC range of 0.25–8 μg/ml with a mode of 1 μg/ml for both *S. aureus* and the CoNS. The vancomycin MIC range from the Vitek was ≤0.5–32 μg/ml with a mode of ≤0.5 μg/ml for *S. aureus* and 2 μg/ml for CoNS. The Vitek did not report vancomycin MICs of 1 or 4 μg/ml for any of the study isolates.

On initial testing by BMD, five isolates of *S. aureus* and five of CoNS demonstrated vancomycin MICs of 8 μg/ml (Figure 1). However, by Vitek testing, vancomycin MICs of 8 μg/ml were reported for only two isolates (one *S. aureus* and one CoNS), although for 19 isolates (12 *S. aureus* and seven CoNS) the vancomycin MICs were 16 μg/ml. Vancomycin MICs of ≥32 μg/ml were reported initially for two *S. aureus* isolates, but the MICs decreased to ≤0.5 μg/ml on retesting (Figure 1).

Of the 23 strains for which the vancomycin MICs were 8 to ≥32 μg/ml by Vitek, only nine showed MICs of 8 μg/ml by BMD; for the remainder MICs were 2–4 μg/ml. The two strains for which the initial Vitek vancomycin

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Table 1

<table>
<thead>
<tr>
<th>Staphylococcus spp.</th>
<th>No. of isolates</th>
<th>MIC Results (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Broth microdilution method</td>
<td>Vitek (GPS-107)</td>
</tr>
<tr>
<td></td>
<td>≤0.06</td>
<td>0.12</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>84</td>
<td>——</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>32</td>
<td>——</td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>8</td>
<td>——</td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>7</td>
<td>——</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>6</td>
<td>——</td>
</tr>
<tr>
<td><em>S. saprophyticus</em></td>
<td>2</td>
<td>——</td>
</tr>
<tr>
<td><em>S. lugdunensis</em></td>
<td>2</td>
<td>——</td>
</tr>
<tr>
<td><em>S. capitis</em></td>
<td>1</td>
<td>——</td>
</tr>
<tr>
<td><em>S. simulans</em></td>
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<td>——</td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>0</td>
</tr>
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</table>

* The vancomycin MICs for these isolates dropped to ≤0.5 μg/ml on retesting.
MICs were ≥32 μg/ml had BMD MIC results of 1 μg/ml (which is consistent with the MICs of ≤0.5 μg/ml observed after retesting the strains by Vitek). When the vancomycin MIC results from the Vitek and BMD were compared using BMD as the gold standard, the MIC results for 49 (34.3%) isolates were in absolute agreement, 77 (53.8%) were in essential agreement (+/− one dilution), 14 (9.8%) had a two dilution difference, and three (2.1%) differed by three or more dilutions. Of the 17 MIC comparisons (13 S. aureus, two S. haemolyticus, and two S. epidermidis) that varied by two dilutions or more, two (those with initial MICs of ≥32 μg/ml) constituted major errors, 13 were minor errors, and two did not result in categorical errors. The percentage of MIC results for oxacillin-susceptible staphylococci that were classified as categorical errors (10.0%) was similar to the percentage of MIC results for oxacillin-resistant staphylococci that were classified as having categorical errors (10.4%).

Fig. 1. Comparison of MIC (μg/ml) results for broth microdilution (BMD) and Vitek methods for 143 staphylococcal isolated tested in phase I. Solid lines indicate MIC breakpoints for interpretive categories (susceptible, intermediate, resistant). Area between dashed lines indicates MIC results that are +/− 1 dilution from BMD results. Bold underlined numbers represent major errors. Numbers in bold (not underlined) represent minor errors. For the two strains marked with an asterisk, MICs were ≤0.5 μg/ml when retested on the Vitek.
3.2 GPS-105 versus GPS-107 cards (Phase II)

For 139 isolates, the MIC results obtained using GPS-105 cards were compared with those using GPS-107 cards (Figure 2). Absolute agreement was shown for MIC results from 129 (92.8%) of the isolates; results for two (1.4%) additional isolates showed essential agreement. For the latter two isolates the GPS-105 card yielded MICs of 16 μg/ml for both isolates while the GPS-107 card reported one isolate as 32 μg/ml and the other as 8 μg/ml. Repeat testing of these isolates on the Vitek using GPS-105 and GPS-107 cards demonstrated vancomycin MICs of 16 μg/ml for both isolates while the GPS-107 card reported one isolate as ≥32 μg/ml and the other as 8 μg/ml. Repeat testing of these isolates on the Vitek using GPS-105 and GPS-107 cards demonstrated vancomycin MICs of 16 μg/ml for both isolates. For eight (5.8%) isolates (six S. aureus, one S. hominis, and one S. warneri) the MICs reported by the two cards differed by two dilutions. Among these isolates, the MICs reported for the GPS-105 and the GPS-107 cards were ≤0.5 and 2 μg/ml (four isolates), 2 and 16 μg/ml (two), 2 and ≤0.5 μg/ml (one), and 16 and 2 μg/ml (one), respectively.

3.3 Inter-laboratory comparison of Vitek MIC results (Phase III)

Fifteen isolates from Phases I and II, for which the vancomycin MICs were 1, 4, or 8 μg/ml by BMD, were selected for further testing in hospitals A and B by the Vitek system with software version 7.01 using GPS-106 cards (Table 2). For six of the 15 isolates that demonstrated BMD MICs of 4 μg/ml, hospitals A and B reported MICs by Vitek of 2 μg/ml (five) or 16 μg/ml (one). For the two isolates with BMD MICs of 8 μg/ml, hospital A reported Vitek MICs of 2 μg/ml, but hospital B reported MICs of 16 μg/ml. Neither hospital reported Vitek MICs for vancomycin of 1 μg/ml or 4 μg/ml for any of the study isolates, as was true in Phases I and II of this study.

4. Discussion

Testing staphylococci with reduced susceptibility to vancomycin using automated MIC methods, such as Vitek and Microscan rapid panels, showed poor sensitivity in earlier studies (Tenover et al., 1998). This study assessed the ability of the new Vitek software Version 7.01 to detect reduced susceptibility to vancomycin in staphylococcal isolates. Our data show that the Vitek was able to detect S. aureus and CoNS isolates with reduced susceptibility to vancomycin; however, MIC results generated by Vitek frequently were higher than those reported by BMD. Vitek vancomycin MICs of ≥32 μg/ml were not reproducible. We recommend that all isolates for which the vancomycin MICs are ≥4 μg/ml by Vitek testing should be repeated. If the high MICs from Vitek are reproducible, the isolates should be tested using an alternative NCCLS-approved method, such as BMD using a 24-h incubation period, to confirm the resistance phenotype.

Comparison of the vancomycin MIC data between the GPS-105 and GPS-107 cards showed no systematic differences. Therefore, the decision to use the GPS-105 or GPS-107 card can be based on other considerations, such as the
variety of other antimicrobial agents that each card has to offer.

Because Vitek software version 7.01 did not report vancomycin MICs of 1 or 4μg/ml, the distribution of vancomycin MICs for the Vitek method was quite different from that of the BMD method, and different from those produced by previous versions of the Vitek software in our laboratory. This may result, over time, in an upward shift of vancomycin MICs in a healthcare facility’s antibiogram. Although this shift may not change the categorical interpretation of the individual susceptibility test results, the increase may erroneously suggest increasing resistance to vancomycin in a healthcare facility.

Notes
1. Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

Acknowledgments
We thank Robert Jerris and David Lonsway for their testing of selected isolates and Christine Steward for her advice and helpful discussions.

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References

Table 2
Phase III: Vitek vancomycin MIC (μg/ml) comparison for fifteen S. aureus isolates tested at three sites

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Emory/CDC Phase I</th>
<th>Emory/CDC Phase II</th>
<th>Phase III</th>
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<tr>
<td></td>
<td>Broth microdilution</td>
<td>Vitek GPS-107</td>
<td>Vitek GPS-105</td>
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<tr>
<td>HIP 5173</td>
<td>4</td>
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