Vancomycin minimum inhibitory concentrations using different susceptibility methods in *Staphylococcus aureus* isolates

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*Staphylococcus aureus* is one of the most common causes of bloodstream infections (BSI) [1] and vancomycin has been used as the main therapy to treat methicillin-resistant *S. aureus* (MRSA) BSI [2]. Although commercial methods are frequently used in clinical laboratories, some studies have shown that their minimum inhibitory concentrations (MIC) values are higher when compared to the broth microdilution (BMD) method, recommended by the Clinical and Laboratory Standards Institute (CLSI), which can lead to erroneous treatment [3]. Besides, reduced MIC for vancomycin among clinical *S. aureus* isolates have already been reported [4]. The present study aimed to verify the effectiveness of three different methods used to detect vancomycin MIC values in *S. aureus* isolates recovered from BSI collected in two hospitals in Rio de Janeiro, between January 2008 and July 2009.

A total of 124 *S. aureus* isolates from BSI, collected in two tertiary-care public hospitals in Rio de Janeiro, hospital 1 (58 isolates) and hospital 2 (66), were recovered. The isolates were identified by automated methods and phenotypic tests [5]. Besides, all isolates were submitted to disk diffusion test (DD), as recommended by CLSI. Vancomycin (Sigma-Aldrich, St. Louis, USA) susceptibility was assessed by the vancomycin-screening method at 3µg/mL [6] and 6µg/mL, and the MIC determination was carried out by the BMD and the agar dilution method (AD), according to CLSI instructions. The Etest (Ab Biodisk, Solna, Sweden) was also used following the manufacturer’s instructions for vancomycin. The Fisher’s exact test and chi-square test were used to compare the data. Significance was established at 5% (p < 0.05).

According to the DD method, 29% and 42% of isolates were resistant to the cefoxitin disk in hospital 1 and hospital 2, respectively, and classified as MRSA isolates. Besides, *S. aureus* isolates from hospital 2 showed higher rates of resistance for erythromycin, ciprofloxacin, mupirocin, clindamycin and chloramphenicol when compared to isolates from hospital 1 (p < 0.05). Moreover, four isolates recovered from hospital 2 were resistant to linezolid.

The vancomycin MIC values for the three methods are shown in Table 1. For the BMD method, 77 (62%) isolates showed a MIC of 0.5 µg/mL and 47 (38%) presented a MIC of 1 µg/mL. When evaluated by the AD and Etest methods, 116 (93.5%) isolates and 54 (44%) isolates displayed a MIC value of 1 µg/mL respectively for each test. Moreover a MIC value ≥ 1.5 µg/mL was detected for 70 (56%) and 2 (1.8%) isolates for the Etest and AD methods, respectively. In addition, the Etest method detected two isolates with vancomycin MIC value of 3 µg/mL.

When the dilution difference in vancomycin MIC value obtained for the AD test was compared with the results of the BMD reference method, 51 (41%) *S. aureus* isolates were detected as concordant (Table 2). However, only 12 (10%) isolates were concordant...
with the values obtained by the BMD reference method when the isolates were tested with the Etest. The AD method revealed 73 (58.9%) isolates with MIC values two times (1 log₂ concentration) higher than those tested by the BMD method, whereas the Etest method showed 37 (29.8%) isolates with MIC values four times (2 log₂ concentration) higher than those evaluated by the BMD, showing that the Etest tended to give higher MIC values.

In the present study, 45 (36%) S. aureus isolates recovered from BSI were characterized as MRSA, according to the cefoxitin disk diffusion test. Different from our findings, some studies conducted in China and India had shown higher rates of MRSA isolates [7,8], whereas in Brazil the resistance rate has remained around 40% [1]. Comparing the results obtained with the susceptibility test for 14 antimicrobials from isolates from the two hospitals, it was possible to detect more MRSA isolates causing BSI in hospital 2 than in hospital 1. Besides, higher rate of resistance was found among hospital 2 isolates, mainly for the antimicrobials ciprofloxacin, clindamycin, erythromycin, chloramphenicol and mupirocin. Differences in resistance rates reported in literature have been correlated to differences in the use of certain antimicrobials in health care institutions [9]. Additionally, four isolates in the present study were considered resistant to linezolid. The occurrence of linezolid-resistant staphylococci has been reported worldwide [10]. In 2006, Gales et al. [11] reported the first case of linezolid-resistant S. aureus isolate in our country.

All 124 S. aureus isolates from BSI from the two hospitals in the city of Rio de Janeiro were considered susceptible to vancomycin by BMD. None of the isolates grew at the vancomycin-screening test. However, when other methods to detect susceptibility for vancomycin were used the MIC values were significantly higher for both the AD method (p = 0.031) and the Etest method (p = 0.002).

Prakash et al. [12] analyzed 101 samples of S. aureus from BSI and detected 76% and 87% of isolates presenting MIC of 1 µg/mL to vancomycin by using the BMD and AD tests, respectively. However, 89 to 98% of vancomycin MICs were 1.5 or 2 µg/ml by using the Etest and only 3 to 12% were 2 µg/ml when determined by the BMD or AD methods. Likewise, we found in the present study that the AD method, but specially the Etest tended to show higher MIC values for vancomycin.

It is commonly accepted that susceptibility test results can vary by one dilution in relation to the reference method [13,14]. However, in the present study, vancomycin Etest MICs tended to be higher than those produced by the BMD method, presenting some values with two dilutions higher. Swenson et al. [14] compared commercial tests with the BMD method to analyze 129 S. aureus isolates from the Center for Disease Control and Prevention (CDC), and found vancomycin MICs ranging from ≤ 1 µg/mL to 8 µg/mL, showing that the Etest method erroneously categorized susceptible isolates as vancomycin-intermediate S. aureus (VISA). As showed in the study, the authors found a higher agreement between the BMD and the AD method (79.8%) than between the BMD and the Etest method (60%). Other studies have shown that the Etest MICs tend to be higher than the BMD MICs [3, 13]. Lodise et al. [15] suggested that there is a relationship between vancomycin treatment failure or worsening of clinical outcome

\[ \text{Table 1. Vancomycin MICs determined by three different methods in 124 Staphylococcus aureus isolates from bloodstream infections} \]

<table>
<thead>
<tr>
<th>Method</th>
<th>No (%) of results with vancomycin MIC (µg/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>BMD*</td>
<td>77 (62%)</td>
</tr>
<tr>
<td>AD⁰</td>
<td>6 (4.8%)</td>
</tr>
<tr>
<td>Etest</td>
<td>0</td>
</tr>
</tbody>
</table>

* BMD: broth microdilution; ⁰ AD: agar dilution.

\[ \text{Table 2. Dilution difference of two test methods compared with the results obtained by the broth microdilution reference method} \]

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of results with dilution difference of *:</th>
<th>% of results with dilution difference of ±1b:</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD⁰</td>
<td>-2</td>
<td>-1</td>
</tr>
<tr>
<td>Etest</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* -2: result by the test method is 2 log₂ concentrations lower than the Broth microdilution (BMD) MIC; -1: result by the test method is 1 log₂ concentration lower than the BMD MIC; 0: result by the test method is the same as the BMD MIC; +1: result by the test method is 1 log₂ concentration greater than the BMD MIC; +2: result by the test method is 2 log₂ concentrations greater than the BMD MIC; b Essential agreement; AD: agar dilution.
with increasing vancomycin MICs. Therefore, clinicians might take into consideration the susceptibility testing method to choose the antimicrobial chemotherapy.

The findings demonstrated that vancomycin MICs in *S. aureus* isolates from BSI were ≤ 1 µg/mL when the BMD reference method was used. However, the agar dilution test, but specially the Etest erroneously detected isolates with higher MICs, which could lead to the unnecessary use of alternative therapies.

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**References**


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