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# Comparative evaluation of the Phoenix®, VITEK® 2, E-test® and microdilution test for vancomycin susceptibility testing in *Staphylococcus aureus* isolated from bloodstream infection

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## Abstract

Introduction: *Staphylococcus aureus* bacteremia causes significant morbidity and mortality, mainly by methicillin-resistant S. *aureus* (MRSA). Currently, vancomycin is the main choice for the treatment of infections by MRSA. Broth microdilution (BMD) remains the gold standard for measuring vancomycin MIC. However, most clinical laboratories employ practical methods in the routines, but these methods may not determine accurate vancomycin MIC values. Objectives: This study aimed to evaluate the accuracy of VITEK®2, Phoenix® and Etest® methods against BMD. Materials and Methods: A total of 78 strains (27 methicillin-sensitive *S. aureus* and 51 MRSA) were isolated from bloodstream infections. The vancomycin MIC was determined following CLSI and the manufacturers' recommendations. We also performed SCC*mec* typing, in order to identify their vancomycin MIC ratio values. Results: Most of all isolates showed values of MIC = 1 µg/mL by BMD and Phoenix®, while Etest® and VITEK® 2 determined the majority with MIC = 1.5 and 0.5 µg/mL, respectively. Thus, Etest® and VITEK® 2 tended to overestimate and underestimate, respectively, the MIC values. Three MRSA isolates that were vancomycin susceptible by the BMD were vancomycin-intermediate by Etest®. The SCC*mec* II (39%) and IV (51%) were the most frequent, and there was no relationship between the type of SCC*mec* and the MIC values. Conclusions: The results showed that vancomycin MICs vary according to the test method. It is essential that clinicians consider the differences in MIC results determined by different methods, since the MIC value is generally the parameter used by clinicians to select the appropriate therapy.

**Descritores:** *Staphylococcus aureus*; Vancomycin; Bloodstream infection.

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## Introduction

*Staphylococcus aureus* is a leading cause of bacteremia, with an estimated mortality of 20%.[1] Bacteremia caused by methicillin-resistant *S. aureus* (MRSA) is associated with poorer clinical outcomes, showing morbidity and mortality higher than methicillin-sensitive *S. aureus* (MSSA) bacteremia.[2]

The resistance to methicillin is mediated by acquisition of the *mecA* gene, which is located within the mobile genetic element Staphylococcal Cassette Chromosome *mec* (SCC*mec*). Currently, many types of SCC*mec* have been described; among these, SCC*mec* types I–V are the most frequently reported.[3,4]

The recommended antimicrobials for the treatment of bacteremia caused by MSSA include β-lactamase-resistant penicillins such as methicillin, and vancomycin is the standard first-line treatment for MRSA bacteremia.[5]

While vancomycin non-susceptible strains remain rare, an increasing proportion of MRSA isolates with high MICs have been observed within the susceptible range around de world, including Brazil (vancomycin MIC creep).[6,7] However, the interpretation of literature on vancomycin MIC creep is complicated by inconsistencies of susceptibility testing methods.[8] It has been suggested that there is an increased risk of treatment failure in MRSA bacteremia caused by strains with reduced vancomycin susceptibility,[9,10] although conflicting results have been published.[11,12] The differences

of these findings can be explained by the methodology performed to determine MIC, because the differences in methodology are well known to affect the MIC value and, simultaneously, can have significant consequences for patients.[8,13]

Different methodologies are available to measure vancomycin MICs. According to CLSI,[14] broth microdilution (BMD) is considered the gold standard for measuring vancomycin MIC. However, BMD is commonly regarded as a laborious and expensive method that is rarely performed in routine diagnostics.[15] Therefore, most clinical laboratories use strips with antimicrobial concentration gradient and automated systems to perform susceptibility testing to determine vancomycin MIC. However, these alternative methodologies to BMD may not be sufficiently accurate, compromising the patients' clinical outcome, since the determination of vancomycin MIC can influence the agent used to treat MRSA infection.[16,17]

The determination of the MIC value is generally the parameter used by clinicians to select the appropriate therapy, which further emphasizes the importance of an accurate MIC value. Thus, a more robust evaluation of the alternative methodologies to BMD is needed, since it influences the patients' clinical outcome.[18] In addition, specific organism characteristics, for example, SCC*mec* types, are linked with elevated vancomycin MIC.[19]

Therefore, the aim of this study was to evaluate the accuracy of several methods in the determination of vancomycin MIC among clinical MRSA/MSSA isolate from bloodstream compared against a BMD standard. We also performed SCC*mec* typing in order to identify their vancomycin MIC ratio values.

## Materials and methods

### Bacterial isolates

A total of 78 *S. aureus* strains, including 51 MRSA and 27 MSSA, isolated from bloodstream were performed; only one isolate per patient was included. All isolates were obtained from patient monitoring at Hospital Universitário Pedro Ernesto (HUPE) of the Universidade do Estado do Rio de Janeiro, over a period of three years. The isolates were identified by VITEK® 2 (GP ID card, BioMérieux, France) as *S. aureus* and were confirmed by classical methodology.20 Resistance to methicillin was performed using cefoxitin (Becton, Dickinson and Company, Sparks, USA) by disk diffusion test (CLSI, 2019).[14]

### Vancomycin MIC determination

Vancomycin susceptibility testing was performed by microdilution test, as a gold standard (CLSI, 2019).[14] MIC was also evaluated by commercial methodologies: Phoenix® (BD Diagnostics, United Kingdom) version V6.21A, the panel type PMIC/ID-89 with vancomycin range from 0.5 to 16 μg/mL, VITEK® 2 (BioMérieux, France) software version 06.01, the card type AST-P585 with vancomycin dilutions range from 1 to 16 μg/mL, and Etest® (BioMérieux, France) gradient strips range from 0.016 to 256 μg/mL.

*S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were applied as control quality.

### Molecular typing (SCC*mec*)

Molecular characterization of SCC*mec* (types I – V) was carried for all MRSA isolates, by multiplex PCR analysis, as previously described.[21]

### Statistical analysis

Comparisons between groups were made with descriptive statistics, on the basis of mean, standard deviation, and coefficient of variation. Before statistically testing, the Kolmogorov-Smirnov test was used on evaluating the variables for normal distribution. Because the samples were not normally distributed, statistical analysis was performed using the non-parametric Kruskal–Wallis test and Dunn's post-test for comparisons between groups. Differences were considered significant at $p < 0.05$. Statistical analyses were performed with GraphPad Prism version 8.0 (GraphPad Software Inc., San Diego, USA). In addition, the concentration required to inhibit 50% ($MIC_{50}$) and 90% ($MIC_{90}$) of the isolates were calculated for each method. In comparison with BMD, categorical agreement between the test methods was assessed as per CLSI14 breakpoint (≤ 2 μg/mL) and suggested breakpoint by the clinical good outcome studies of ≤1 μg/mL.[22,23]

## Results

Table 1 shows vancomycin MIC values for the four methods. Overall, by BMD, all except one isolate (MIC = 2 μg/mL) had MICs ≤ 1 μg/mL. Similarly, all isolates had MICs ≤ 1 μg/mL by VITEK® 2. On the other hand, Phoenix® and Etest® had MIC values > 1 μg/mL, with a frequency of 9% and 63% in all isolates tested, respectively. In the MSSA group, only Etest® determined MIC values > 1 μg/mL. Notably, all isolates determined with MIC > 1 μg/mL by BMD and Phoenix® belonged to the

MRSA group. Altogether, BMD and Phoenix® tended to determine values of MIC = 1 µg/mL, while VITEK® 2 and Etest® determined the majority with MIC = 0.5 µg/mL and 1.5 µg/mL, respectively. Three MRSA isolates that were vancomycin susceptible by the BMD method were vancomycin-intermediate by Etest® (MIC > 2 µg/mL). All isolates had an $MIC_{50}$ and $MIC_{90}$ of ≤ 1 µg/mL for BMD, Phoenix® and VITEK® 2, except the MRSA group for the Phoenix®, which had $MIC_{90}$ of 2 µg/mL. On the other hand, the Etest® was the only method that had values of $MIC_{50}$ and $MIC_{90}$ > 1 µg/mL.

In the MRSA group, the mean Phoenix® vancomycin MIC was 1.14 µg/mL, value closer to BMD (1.01 µg/mL). The MICs determined by the VITEK® 2 were frequently lower, showing lower mean values. The Etest® had a higher disagreement with the means of MICs by BMD in the three groups (all strains, MSSA and MRSA), while the Phoenix® system had values closer to the BMD coefficient of variation. The Etest® presented higher values for the coefficient of variation, showing that there was high variability of the MIC values in relation to the reference method (Table 2).

Categorical agreements for the three methods, compared to BMD, are shown in Table 3. VITEK® 2 and Phoenix® showed a high level of agreement between breakpoints with the gold-standard BMD assay MICs. Etest® had a good agreement only on breakpoint ≤ 2 µg/mL.

Among the 51 MRSA isolates, 2 (4%) harbored the SCC*mec* I, 20 (39%) the SCC*mec* II, 1 (2%) the SCC*mec* III, 26 (51%) the SCCmec IV and 1 (2%) the SCC*mec* V. It was not possible to determine the SCC*mec* type of one isolate (MRSA)(2%).

Significant differences (Kruskal-Wallis test, P < 0.0001) were detected among the four methods, in the three groups of isolates (all strains, MSSA and MRSA). Dunn's nonparametric pairwise comparison, testing after a significant Kruskall–Wallis, was calculated using BMD as the reference method. Thus, in all groups (all strains, MSSA and MRSA), significant differences (P < 0.05) were observed between the BMD and VITEK®

**Table 1. Distribution of vancomycin MICs, according to the method**

| | No. of Isolates (%) inhibited at MIC (µg/mL) of: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Method** | **≤ 0.5** | **1** | **1.5*** | **2** | **3*** | **4** | **$MIC_{50}$** | **$MIC_{90}$** |
| All strains (n = 78) | | | | | | | | |
| Broth microdilution | 12(15.4) | 65(83.3) | - | 1(1.3) | - | - | 1 | 1 |
| Phoenix® | 3(3.8) | 68(87.2) | - | 7(9) | - | - | 1 | 1 |
| VITEK® 2 | 65(83) | 13(17) | - | - | - | - | 0.5 | 1 |
| Etest® | 5(6) | 24(31) | 32(41) | 14(18) | 1(1) | 2(3) | 1 | 1.5 |
| MSSA (n = 27) | | | | | | | | |
| Broth microdilution | 11(41) | 16(59) | - | 1(1.3) | - | - | 1 | 1 |
| Phoenix® | 3(11) | 24(89) | - | 7(9) | - | - | 1 | 1 |
| VITEK® 2 | 23(85) | 4(15) | - | - | - | - | 0.5 | 1 |
| Etest® | 4(15) | 10(37) | 11(41) | 2(7) | - | - | 1 | 1.5 |
| MSSA (n = 51) | | | | | | | | |
| Broth microdilution | 1(2) | 49(96) | - | 1(2) | - | - | 1 | 1 |
| Phoenix® | - | 44(86.3) | - | 7(13.7) | - | - | 1 | 2 |
| VITEK® 2 | 42(82) | 9(18) | - | - | - | - | 0.5 | 1 |
| Etest® | 1(2) | 14(27) | 21(41) | 22(24) | 1(2) | 2(4) | 1.5 | 2 |

Legend: MRSA: Methicillin-Resistant *Staphylococcus aureus*; MSSA: Methicillin-Susceptible *S. aureus*; MIC: Minimum Inhibitory Concentration; $MIC_{50}$: concentration that inhibited growth of 50% of isolates; $MIC_{90}$: concentration that inhibited growth of 90% of isolates; * concentration present only on the Etest®.
Authorship: The authors (2020).

**Table 2. Descriptive statistics of all MICs determined by each method**

| Method | Mean (µg/mL) | Standard deviation | Coefficient of variation |
|---|---|---|---|
| All strains (n = 78) | | | |
| Broth microdilution | 0.94 | 0.22 | 23.5 |
| Phoenix® | 1.1 | 0.31 | 28.9 |
| VITEK® 2 | 0.59 | 0.19 | 32.9 |
| Etest® | 1.46 | 0.62 | 42.3 |
| MSSA (n = 27) | | | |
| Broth microdilution | 0.80 | 0.25 | 31.44 |
| Phoenix® | 0.94 | 0.16 | 16.95 |
| VITEK® 2 | 0.57 | 0.18 | 31.53 |
| Etest® | 1.20 | 0.42 | 35.05 |
| MSSA (n = 51) | | | |
| Broth microdilution | 1.01 | 0.16 | 15.62 |
| Phoenix® | 1.14 | 0.35 | 30.56 |
| VITEK® 2 | 0.60 | 0.20 | 33.78 |
| Etest® | 1.59 | 0.67 | 41.89 |

Legend: MRSA: Methicillin-Resistant *Staphylococcus aureus*; MSSA: Methicillin-Susceptible *S. aureus*; MIC: Minimum Inhibitory Concentration.
Authorship: The authors (2020).

**Table 3. Categorical agreement between Phoenix®, VITEK® 2 and Etest® compared to broth microdilution method**

| Breakpoints (µg/mL) | Agreement (%) All strains | Agreement (%) MSSA | Agreement (%) MRSA |
|---|---|---|---|
| Phoenix® | | | |
| ≤ 2 | 100 | 100 | 100 |
| ≤ 1 | 91 | 100 | 86.3 |
| VITEK® 2 | | | |
| ≤ 2 | 100 | 100 | 100 |
| ≤ 1 | 98.7 | 100 | 98 |
| Etest® | | | |
| ≤ 2 | 96.1 | 92.6 | 94.1 |
| ≤ 1 | 33.3 | 51.9 | 31.4 |

Authorship: The authors (2020).

2, and between the BMD and Etest®. No statistically significant differences were observed between the BMD and Phoenix® ($P > 0.05$) (Table 4).

## Discussion

The susceptibility of microorganisms to antimicrobials in clinical microbiology laboratories needs to be simple, reliable, and accurate, since these microorganisms influence therapeutic decision-making. According to CLSI,14 BMD is considered the gold standard to determine the susceptibility of *S. aureus* to vancomycin; however, this procedure is laborious and is not used routinely by clinical laboratories. Most clinical laboratories use gradient MIC strips and automated susceptibility testing to measure vancomycin MIC.[16] Nonetheless, there is a subtle variability in the MIC values of vancomycin obtained with different methodologies, which is a problem, since the MIC value is one of the parameters for choosing the therapy.[24] Thus, reliable methods to test the susceptibility of *S. aureus* to vancomycin are needed to predict the appropriate clinical response.

All the isolates were susceptible to vancomycin

**Table 4. Statistical differences between the three methods compared to broth microdilution method using Dunn's multiple comparison test**

| Method | P-value |
|---|---|
| All strains (n = 78) | |
| Phoenix® | 0.1883 |
| VITEK® 2 | <0.0001 |
| Etest® | <0.0001 |
| MSSA (n = 27) | |
| Phoenix® | 0.2044 |
| VITEK® 2 | 0.0187 |
| Etest® | 0.0007 |
| MRSA (n = 51) | |
| Phoenix® | 0.7435 |
| VITEK® 2 | <0.0001 |
| Etest® | <0.0001 |

Authorship: The authors (2020).

(MIC ≤ 2 μg/mL) by BDM, Phoenix® and VITEK® 2; except for Etest®, which determined three isolates with MIC > 2 μg/mL. In this study, in all strains (n = 78) vancomycin MICs obtained by Etest® were consistently higher (mean 1.46 μg/mL), while VITEK® 2 had lower MIC values (mean 0.59 μg/mL), when compared with BDM (mean 0.94 μg/mL), demonstrating that the Etest® and VITEK® 2 tend to overestimate and underestimate the value of MIC, respectively. The vancomycin MIC values obtained by Phoenix® (mean 1.1 μg/mL) correlated better with BMD method.

A study in the USA has made a comparative analysis between commercial methodologies, including Phoenix®, Etest® and VITEK® 2, with the standard BMD methodology for measuring MIC vancomycin of 200 MRSA isolated from blood, 10 more control strains.25 In their study, the results showed that 60% of the isolates submitted to Etest® presented MICs with 1 more dilution than the MICs determined by BMD; while VITEK® 2 and the Phoenix® systems were more likely to underestimate the MIC by 1 dilution at 32.3% and 26.7% of isolates, respectively. Similar to the current study, VITEK® 2 and Etest® also tended to underestimate and overestimate MIC values in 74% (58/78) and 68% (58/78) of the isolates, respectively; however, the Phoenix® system tended to overestimate MIC in 20% (18/78) of isolates. Likewise, Rybak et al., in their study also reported that Phoenix® (66.2%) was the method with the highest agreement with the MIC values by the reference method.[25]

In the current study, we showed that the MICs determined by VITEK® 2 tends to present values of 0.5 μg/mL (65/78, 83%), while Etest® tended to result values > 1 μg/mL (49/78, 63%), disagreeing with the gold standard methodology, which tended to report values equal to 1 μg/mL (65/78, 83.3%). Similar to BDM, the Phoenix® system tended to have MIC equal to 1 μg/mL (68/78, 87.2%). A study also reported that the majority of vancomycin MICs determined by VITEK® 2 and Etest® had values of 0.5 μg/mL and 1.5 μg/mL, respectively; however, the Phoenix® system tended to determine MIC of 0.5 μg/mL.[26] Reports are conflicting about the performance of the Phoenix® system compared to the standard BMD method.[27]

The only isolate (MRSA) with MIC = 2 μg/mL by BDM was determined with MIC of 1 μg/mL by VITEK® 2 and Phoenix®, and when tested with Etest®, the isolate had MIC of 3 μg/mL. A previous study that determined the vancomycin susceptibility of 129 *S. aureus* isolates from the CDC (Centers for Disease Control and Prevention) collection showed that Etest® and the Phoenix® system tended to categorize VSSA (vancomycin-susceptible *S. aureus*) strains as VISA (vancomycin-intermediate *S. aureus*), while VITEK® 2 tended to categorize VISA strains as VSSA.[28] Changes in vancomycin MIC, even if small, could have relevant consequences for patients, due to the narrow therapeutic window of vancomycin or the incorrect categorization of MIC, such as determining VSSA a strain that is VISA.[8]

Our results show the risk of the VITEK® 2 automated system in underestimating MIC values, especially when there are VISA subpopulations (hVISA - heteroresistant vancomycin-intermediate *S. aureus*). Underestimating MIC values can lead to severe therapeutic results or risk of failure, since clinicians do not normally change therapy for isolates with MICs ≤ 1 μg/mL of vancomycin.[25] Vancomycin MIC in MRSA with values between 1-2 μg/mL is more likely to result in treatment failure with vancomycin due to the possible presence of VISA subpopulations, considering alternative therapies in patients with persistent infections, such as bacteremia. Since hVISA is associated with poor results, it is of great importance that a methodology has the capacity to distinguish MIC values between 1 and 2 μg/mL of vancomycin.[29,30]

If the cut-off point for *S. aureus* susceptibility to vancomycin was adjusted down to ≤ 1 μg/mL, which is the cut-off point suggested by the studies for better clinical results,[22] only 37% of the isolates (29/78) would be categorized as VSSA by Etest®. In contrast, 98.7% and 100% of the isolates submitted to Phoenix® and

VITEK® 2, respectively, would be categorized as VSSA. Thus, possibly, less than half of the patients would be indicated to start treatment with vancomycin when using Etest® to determine MIC; conversely, if using Phoenix® and VITEK® 2 systems, all patients would have a high chance of starting treatment with vancomycin. Similar results were found in a previous study, in 359 isolates (MRSA and MSSA), which when assuming MIC ≤ 1 µg/mL, 96% and 16% of patients, respectively, would receive treatment with vancomycin when using VITEK® 2 and Etest® to determine the vancomycin MIC.[31]

Vancomycin MICs generated by Etest® were consistently higher in the current study, according to other studies showing that Etest® MICs tend to be higher than BMD MICs.[16,32,33] The falsely elevated MIC of vancomycin may influence the clinicians to use another antimicrobial to replace vancomycin, and in another scenario, with the precise MIC value, vancomycin would be the first treatment choice.[34] Although new anti-staphylococcal antimicrobials have recently been developed, such as linezolid, vancomycin still remains the main therapeutic choice, mainly due to the low cost and the large number of clinical trials when compared to the new drugs.[35]

Only 23.1% (18/78) and 26.9% (21/78) isolates agreed with the values obtained by the BMD reference method when the isolates were tested with the VITEK® 2 and Etest®, respectively. However, when tested with Phoenix®, 78.2% of the isolates were concordant with the values obtained by the BMD. There is no fully reliable method for determining the vancomycin MIC; however, BMD remains the apparently most reliable method; yet, BMD is not routinely used in clinical laboratories. This method has a two-fold serial dilution method, which may not be able to detect subtle changes in the vancomycin MIC.[36] In this study, Phoenix® is a better method to determine vancomycin MICs compared to VITEK® 2 and Etest®. However, the vancomycin MIC values consistently higher by Etest® appear to be more reliable in predicting response to vancomycin treatment.[37,38] Etest® is based on a continuous gradient with half-dilution values (i.e. 1.5 µg/mL). It is suggested to measure isolates with elevated vancomycin MIC (within susceptible range) with a second alternative method, such as Etest®; thus assisting recommendations for antibiotic therapy.[36-38]

The majority of the MRSA isolates carried SCC*mec* type II (n= 20; 39%) or type IV (n= 26; 51%). Likewise, a study carried out at a Brazilian university hospital, which typed 31 isolates from patients with MRSA bacteremia, showed that 48% and 52% of the isolates, respectively, were classified as SCC*mec* II and IV.[39] There was no association with a high vancomycin MIC and the type of SCC*mec*, since 99% of MICs by BMD had values ≤ 1 µg/mL. Nevertheless, 36 MRSA isolates (36/51) had MIC > 1 µg/mL by Etest®; of these isolates, 44% (16/36) carried SCC*mec* type II. A study with 188 patients with MRSA bacteremia found that isolates with reduced susceptibility to vancomycin (MIC by Etest® > 1 µg/mL) were significantly associated with SCC*mec* II compared to isolates without reduced susceptibility to vancomycin.[19] On the contrary, another study found no associations between SCC*mec* with elevated vancomycin MIC and increased mortality in patients with MRSA bacteremia.[12]

A limitation of our study is that we did not evaluate clinical outcomes among the methodology used in this work. We also did not evaluate the hVISA detection in our isolates. It is known that hVISA interferes negatively in the treatment of patients with bacteremia, and that these isolates are often not detected by the methodologies commonly used to determine MIC, and the use of the population analysis profile-area under the curve (PAP-AUC) method is an expensive and laborious procedure.

## Conclusion

In summary, the results of the current study demonstrate that vancomycin MICs vary according to the test methodology. VITEK® 2 and Etest® tend to underestimate or overestimate, respectively, the value of MIC, differing significantly from BMD ($P < 0.05$). The Phoenix® system showed MIC values closer to BMD, no significant differences found between these methodologies ($P > 0.05$). These methodologies are not equivalent to BMD; therefore, they do not accurately replace the gold standard methodology. Therefore, it is important to have alternatives to measure the results of MRSA isolates with MIC between 1 and 2 µg/mL of vancomycin, in order to detect hVISA isolates. The detection method Etest® for resistance to glycopeptides is considered a reasonable methodology to the PAP-AUC method and simple to detect hVISA/VISA in routine laboratories.[40]

It is essential that clinicians consider the differences in MIC results determined by different BMD substitute methodologies, and that awareness of the type of methodology used in the clinical laboratory is relevant before considering antimicrobial therapy for severe

MRSA infections. The variability of methodologies in determining the vancomycin MIC and the value of elevated vancomycin MIC, even within the susceptibility range (≤ 2 μg/mL), are still cause for concern.

## Referências


1. Guimaraes AO, Cao Y, Hong K, et al. A prognostic model of persistent bacteremia and mortality in complicated S. aureus bloodstream infection. Clin Infect Dis. 2018;68(9):1502-1511.
2. Adani S, Bhowmick T, Weinstein MP, et al. Impact of Vancomycin MIC on Clinical Outcomes of Patients with Methicillin-Resistant *Staphylococcus aureus* Bacteremia Treated with Vancomycin at an Institution with Suppressed MIC Reporting. Antimicrob Agents Chemother. 2018;62(4):e02512-17.
3. Balakuntla J, Prabhakara S, Arakere G. Novel rearrangements in the staphylococcal cassette chromosome mec type V elements of Indian ST772 and ST672 methicillin resistant *Staphylococcus aureus* strains. PLoS One. 2014;9(4):e94293.
4. Kaya H, Hasman H, Larsen J, et al. SCCmecFinder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome mec in *Staphylococcus aureus* Using Whole-Genome Sequence Data. mSphere. 2018;3(1)e00612-17.
5. Kalimuddin S, Chan YFZ, Phillips R, et al. A randomized phase 2B trial of vancomycin versus daptomycin for the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia due to isolates with high vancomycin minimum inhibitory concentrations – results of a prematurely terminated study. Trials. 2018;19(1):305.
6. Zuma AVP, Lima DF, Assef APDAC, et al. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from blood in Rio de Janeiro displaying susceptibility profiles to non-β-lactam antibiotics. Brazilian J Microbiol. 2017;48(2):237-241.
7. Diaz R, Ramalheira E, Afreixo V, et al. Evaluation of vancomycin MIC creep in *Staphylococcus aureus*. J Glob Antimicrob Resist. 2017;10:281–4.
8. Edwards B, Milne K, Lawes T, et al. Vancomycin MIC "Creep" Method Dependent? Analysis of Methicillin-Resistant *Staphylococcus aureus* Susceptibility Trends in Blood Isolates from North East Scotland from 2006 to 2010. J Clin Microbiol. 2012;50(2):318–25.
9. van Hal SJ, Lodise TP, Paterson DL. The Clinical Significance of Vancomycin Minimum Inhibitory Concentration in *Staphylococcus aureus* Infections: A Systematic Review and Meta-analysis. Clin Infect Dis. 2012;54(6):755–71.
10. San-Juan R, Fernández-Ruiz M, Gasch O, et al. High vancomycin MICs predict the development of infective endocarditis in patients with catheter-related bacteraemia due to methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother. 2017;72(7):2102–9.
11. de Sanctis JT, Swami A, Sawarynski K, et al. Is there a clinical association of vancomycin MIC creep, agr group II locus, and treatment failure in MRSA bacteremia? Diagn Mol Pathol. 2011;20(3):184–8.
12. Park S-Y, Oh I-H, Lee H-J, al. Impact of Reduced Vancomycin MIC on Clinical Outcomes of Methicillin-Resistant *Staphylococcus aureus* Bacteremia. Antimicrob Agents Chemother. 2013;57(11):5536–42.
13. Hos NJ, Jazmati N, Stefanik D, et al. Determining vancomycin Etest MICs in patients with MRSA bloodstream infection does not support switching antimicrobials. J Infect. 2017;74(3):248–59.
14. CLSI (2019) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-seventh informational supplement. CLSI, M100-S28. Wayne, PA: Clinical and Laboratory Standards Institute.
15. Rossatto FCP, Proenca LA, Becker AP, et al. Evaluation of methods in detecting vancomycin MIC among MRSA isolates and the changes in accuracy related to different MIC values. Rev Inst Med Trop Sao Paulo. 2014;56(6):469–72.
16. Agrawal DC. Methicillin Resistant *Staphylococcus aureus*: Inconsistencies in Vancomycin Susceptibility Testing Methods, Limitations and Advantages of each Method. J Clin DIAGNOSTIC Res. 2015; 9(10):DC01-4.
17. Phillips C, Wells N, Martinello M, et al. Optimizing the detection of methicillin-resistant *Staphylococcus aureus* with elevated vancomycin minimum inhibitory concentrations within the susceptible range. Infect Drug Resist. 2016;9:87-92.
18. Bhatt M, Burgess DR, Wallace KL, et al. Impact of Susceptibility Testing Method on Antibiotic Selection for Methicillin-Resistant *Staphylococcus aureus* (MRSA) Bacteremia. Open Forum Infect Dis. 2018;5(1):511–2.
19. Han JH, Edelstein PH, Lautenbach E. Reduced vancomycin susceptibility and staphylococcal cassette chromosome mec (SCCmec) type distribution in methicillin-resistant *Staphylococcus aureus* bacteraemia. J Antimicrob Chemother. 2012;67(10):2346–9.
20. Menzies RE. Comparison of coagulase, deoxyribonuclease (DNase), and heat-stable nuclease tests for identification of *Staphylococcus aureus*. J Clin Pathol. 1977;30(7):606–8.
21. Boye K, Bartels MD, Andersen IS, et al. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCC mec types I-V. Clin Microbiol Infect. 2007;13(7):725-7.
22. Álvarez R, López Cortés LE, Molina J, et al. Optimizing the Clinical Use of Vancomycin. Antimicrob Agents Chemother. 2016;60(5):2601–9.
23. Toyokawa M, Francisco M, Nishi I, et al. Accuracy of Commercial Susceptibility Testing Method for Measuring Vancomycin MIC Against Methicillin-Resistant *Staphylococcus aureus* (MRSA). Lab Med. 2011; 42(8)473–477.
24. Holmes NE, Johnson PDR, Howden BP. Relationship between Vancomycin-Resistant *Staphylococcus aureus*, Vancomycin-Intermediate S. aureus, High Vancomycin MIC, and Outcome in Serious S. aureus Infections. J Clin Microbiol. 2012;50(8):2548–52.
25. Rybak MJ, Vidaillac C, Sader HS, et al. Evaluation of vancomycin susceptibility testing for methicillin-resistant *Staphylococcus aureus*: Comparison of Etest and three automated testing methods. J Clin Microbiol. 2013;51(7):2077–81.
26. Kruzel MC, Lewis CT, Welsh KJ, et al. Determination of Vancomycin and Daptomycin MICs by Different Testing Methods for Methicillin-Resistant *Staphylococcus aureus*. J Clin Microbiol. 2011;49(6):2272–3.
27. Marx A, Daniels L, Miller MB, et al. Vancomycin minimum inhibitory concentration is not a substitute for clinical judgment: Response to healthcare-associated ventriculitis and meningitis. Clin. Infect. Dis. 2017; 65(8):1428–1429.
28. Swenson JM, Anderson KF, Lonsway DR, et al. Accuracy of

Commercial and Reference Susceptibility Testing Methods for Detecting Vancomycin-Intermediate *Staphylococcus aureus*. J Clin Microbiol. 2009;47(7):2013–7.
29. Liu C, Bayer A, Cosgrove SE, Daum RS, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America for the Treatment of Methicillin-Resistant *Staphylococcus aureus* Infections in Adults and Children. Clin Infect Dis. 2011;52(3):e18–55.
30. van Hal SJ, Paterson DL. Systematic Review and Meta-Analysis of the Significance of Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* Isolates. Antimicrob Agents Chemother. 2011;55(1):405–10.
31. Tomczak H, Szałek E, Błażejewska W, et al. The need to assay the real MIC when making the decision to eradicate *Staphylococcus aureus* with vancomycin. PostepyHig Med Dosw. 2013;67:921–5.
32. Sousa AGP, Da Costa TM, Cavalcante FS, et al. Vancomycin minimum inhibitory concentrations using different susceptibility methods in *Staphylococcus aureus* isolates. J Infect Dev Ctries. 2014;8(04):558–60.
33. Manfredini C, Picoli SU, Becker AP. Comparação de métodos na determinação de sensibilidade à vancomicina em *Staphylococcus aureus* resistente à meticilina. J Bras Patol e Med Lab. 2011;47(2):141–145.
34. Goldman JL, Harrison CJ, Myers AL, et al. No Evidence of Vancomycin Minimal Inhibitory Concentration Creep or Heteroresistance Identified in Pediatric Staphylococcus aureus Blood Isolates. Pediatr Infect Dis J. 2014;33(2):216–8.
35. Ma X, Gao P, Lv X, et al. Vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from 2006 to 2010 in a hospital in China. Indian J Med Microbiol. 2015;33(2):262.
36. Wilcox M, Al-Obeid S, Gales A, et al. Reporting elevated vancomycin minimum inhibitory concentration in methicillin-resistant *Staphylococcus aureus*: consensus by an International Working Group. Future Microbiol. 2019;14(4):345–52.
37. Hsu DI, Hidayat LK, Quist R, et al. Comparison of method-specific vancomycin minimum inhibitory concentration values and their predictability for treatment outcome of meticillin-resistant *Staphylococcus aureus* (MRSA) infections. Int J Antimicrob Agents. 2008;32(5):378–85.
38. Sader HS, Rhomberg PR, Jones RN. Nine-Hospital Study Comparing Broth Microdilution and Etest Method Results for Vancomycin and Daptomycin against Methicillin-Resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2009;53(7):3162–5.
39. da Costa TM, Morgado PGM, Cavalcante FS, et al. Clinical and Microbiological Characteristics of Heteroresistant and Vancomycin-Intermediate *Staphylococcus aureus* from Bloodstream Infections in a Brazilian Teaching Hospital. PLoS One. 2016;11(8):e0160506.
40. Yusof A, Engelhardt A, Karlsson A, et al. Evaluation of a New Etest Vancomycin-Teicoplanin Strip for Detection of Glycopeptide-Intermediate *Staphylococcus aureus* (GISA), in Particular, Heterogeneous GISA. J Clin Microbiol. 2008;46(9):3042–7.