# Accuracy of a vancomycin brain heart infusion screening plate for the screening of *Staphylococcus aureus* isolates with increased vancomycin minimum inhibitory concentrations

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**SUMMARY**

Vancomycin susceptibility was determined in 125 *S. aureus* isolates by disk diffusion, microdilution, Etest and vancomycin brain heart infusion (BHI) plate. A 2.0 mg/L vancomycin BHI was highly sensitive (100% and 91% compared to Etest and microdilution) for detecting a MIC ≥2 mg/L, and could be used as a simple and affordable screening test.

*KEY WORDS:* Vancomycin, Susceptibility, Brain heart infusion, Screening, *Staphylococcus aureus*.

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Antimicrobial-resistant *Staphylococcus aureus* has been an important cause of community and nosocomial infections globally. Vancomycin is frequently used as the therapeutic agent of choice in patients with these infections. Recently, a number of issues have complicated its use, including the occurrence of vancomycin-intermediate (VISA) (Hiramatsu *et al*., 1997) and vancomycin-resistant *S. aureus* (VRSA) (Chang *et al*., 2003), the possibility of a minimum inhibitory concentration (MIC) creep (an overall drift in the vancomycin MICs among susceptible isolates towards non-susceptibility), and evidence showing poor prognosis among patients with increased (although within the "susceptible" range) vancomycin MICs (Wang *et al*., 2006; Tenover and Moellering, 2007; Jones, 2006). Although CLSI currently considers susceptible isolates with an MIC ≤2 mg/L, some authors have demonstrated that infections with isolates with MICSs from 1.5 or 2.0 could be more difficult to treat than infections with isolates with lower MICs (van Hal *et al*., 2012). This could be true not only when the patient is receiving vancomycin, but also when he is being treated with additional antimicrobial agents such as beta-lactams and daptomycin (Holmes *et al*., 2011, 2013). It is important to note that most of these studies used Etest™ to determine MICs.

Much discussion has been invested not only in the clinical use of the drug, but also in how clinical laboratories should deal with these issues. Despite some controversy regarding the exact extension of MIC creep (Sader *et al*., 2009) and the clinical implications of different MIC levels (Wang *et al*., 2006; Tenover and Moellering, 2007; Jones, 2006; van Hal *et al*., 2012; Holmes *et al*., 2011, 2013; Rojas *et al*., 2012), most experts consider that vancomycin MICs could be used at least as an additional parameter (besides and secondarily to clinical parameters) to

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guide antimicrobial therapy (Humphries and Hindler, 2012). Unfortunately, different studies have shown the heterogeneity of the results offered by different laboratory methods. MICs by Etest™ are usually higher than those determined by broth microdilution and even higher than those yielded by automated methods (Mason *et al*., 2009; Vaudaux *et al*., 2010). In addition, most of these tests are relatively expensive or time consuming, and a significant number of clinical microbiology laboratories throughout the world are not able to introduce them into routine practice. There is a need for a simple and inexpensive method that could be used as a primary screening test for reduced susceptibility to vancomycin in *S. aureus*.

This study aimed to evaluate the accuracy of a brain heart infusion vancomycin-supplemented screening plate compared to broth microdilution and Etest™ for the detection of *S. aureus* isolates with possible decreased susceptibility to vancomycin.

We included 125 *S. aureus* isolates from blood cultures of patients hospitalized in a large tertiary-care hospital in São Paulo, Brazil from July 2011 to June 2012. We confirmed the identification of the isolates using biochemical methods and included only one isolate per patient. Susceptibility to vancomycin was determined in all isolates with disk-diffusion, Etest™ (bioMérieux, Marcy l'Etoile, France), broth microdilution (Probac do Brasil, São Paulo, Brasil), and Brain Heart Infusion Agar supplemented with four different vancomycin concentrations (1.5, 2.0, 4.0 and 6.0 mg/L, Probac do Brasil). Results were interpreted according to the Clinical and Laboratory Standards Institute criteria (Clinical and Laboratory Standards Institute, 2013). ATCCs 25923, 29213, 43300 (*S. aureus*), 29212 and 51299 (*E. faecalis*) were used as controls.

All isolates were considered susceptible by disk diffusion and microdilution. The maximum MIC observed was 2.0 (n=55) by microdilution and 4.0 by Etest™ (n=1). No isolates grew on BHI with 6.0 mg/L of vancomycin and only one grew in the 4.0 BHI. Comparing Etest and microdilution (approximating an Etest™ MIC value between two twofold dilutions up to the highest value), 58% (n=73) of the isolates had similar results, whereas 38% (n=47) had an MIC result by Etest™ one dilution higher than microdilution. One isolate had an Etest™ MIC two-fold higher and four isolates an Etest™ MIC onefold lower than microdilution. Etest™ has been consistently demonstrated to produce MIC results higher than broth microdilution and automated methods (Mason *et al*., 2009; Vaudaux *et al*., 2010). Understanding the potential differences in the results yielded by these methods is paramount for the correct interpretation and clinical application of laboratory data.

A minimum inhibitory concentration of 2.0, when compared to a MIC<2.0 was significantly more frequent in MRSA (n=76) than in MSSA (n=49), both by microdilution and Etest ($p$=0,0018 and $p$=0,0001, respectively, by Fisher's exact test). Although higher vancomycin MICs are more common in MRSA than in MSSA, other have also reported reduced susceptibility to vancomycin in MSSA (Holmes *et al*., 2011, 2013; Pillai *et al*., 2009; Aguado *et al*., 2011).

Between the BHI plates with different concentrations of vancomycin, the most accurate was the BHI screening plate with 2.0 mg/L of vancomycin, which had a sensitivity of 100% to detect isolates with an MIC≥2.0 by Etest and 91% to detect an MIC≥2.0 by microdilution, and specificities, respectively of 63% and 38%. For isolates with an MIC≥1.5 by Etest the sensitivity would be 74% and the specificity 65%. The high sensitivity would make this test an interesting option for an initial screening test. The low specificity would make necessary that positive isolates be further tested by Etest or microdilution for confirmation. Others had studied the accuracy of a BHI screening plate (Burnham *et al*., 2010; Riederer *et al*., 2011), but with the primary aim of detecting non-susceptible (VISA) isolates, and not isolates with reduced susceptibility (higher MICs within the susceptibility range), as our study.

Accurate laboratory detection of *S. aureus* with decreased susceptibility to vancomycin has been recommended to help clinicians in the process of decision-making regarding antimicrobial therapy. Different methods have been advocated for this aim, including Etest and broth microdilution, the CLSI reference method. Unfortunately, these methods are not easi-

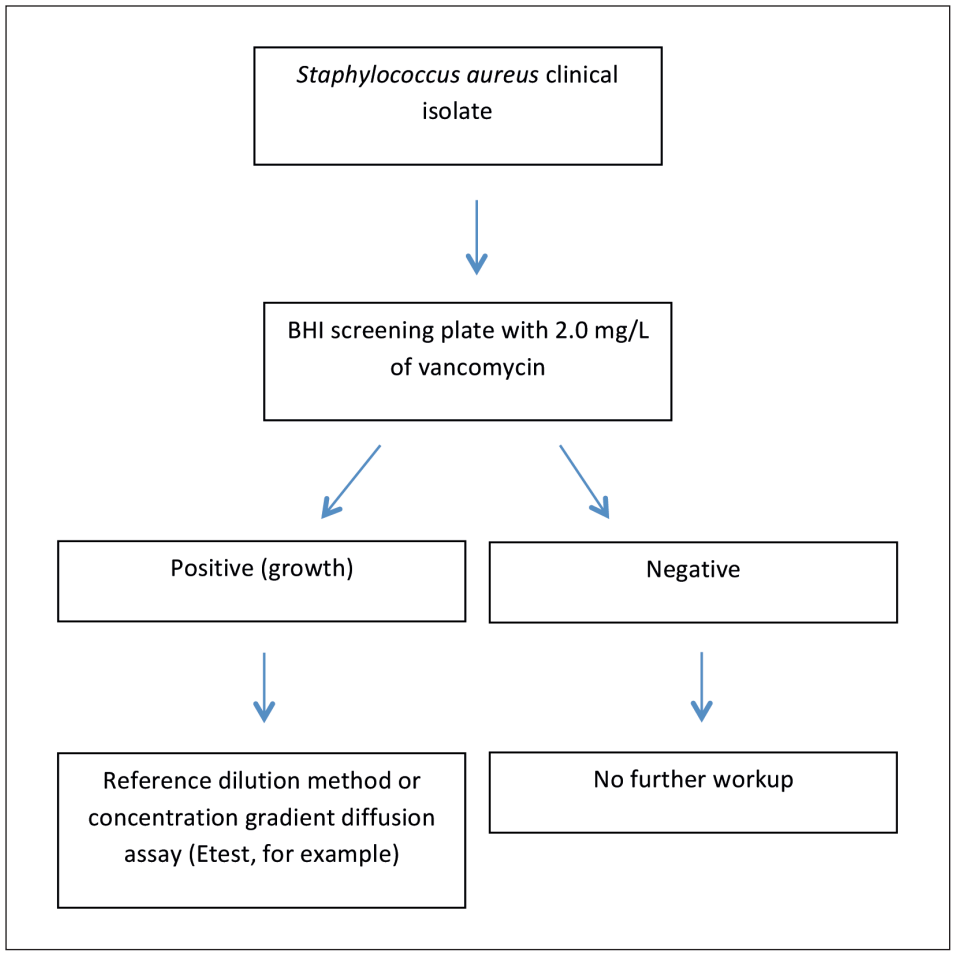


FIGURE 1 - *Workflow for the detection of reduced vancomycin susceptibility in Staphylococcus aureus using BHI screening plate with 2.0 mg/L of vancomycin as a screening method.*

ly available in a significant number of clinical microbiology laboratories globally, and there is still a need for an easy and inexpensive screening test. In a BHI screening plate, various isolates can be tested at the same time, making this method even more practical and affordable. It would be a very suitable option, for example, in laboratories that frequently solely rely on disk diffusion and are not able to determine the MIC in all clinical isolates. We propose (Figure 1) a workflow for such laboratories to detect isolates with reduced vancomycin susceptibility using a BHI screening plate.

Despite some limitations, such as the relatively small number of isolates and the absence of vancomycin-resistant isolates, our results highlight the possibility of using a vancomycin BHI plate as a screening method for the detection of *S. aureus* isolates with decreased susceptibility to vancomycin. Future studies should include a higher number of isolates and resistant/heteroresistant isolates.

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