ORIGINAL ARTICLE

Susceptibility profile of *Staphylococcus aureus* isolates in blood cultures from a tertiary hospital - comparison of methods for determination of minimum inhibitory concentration of vancomycin

Perfil de suscetibilidade de isolados de *Staphylococcus aureus* em hemoculturas de um hospital terciário – comparação de métodos para determinação da concentração inibitória mínima de vancomicina

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Abstract

Objective: to evaluate the antimicrobial susceptibility profile of *Staphylococcus aureus* isolates from blood cultures and compare methods for determining the minimum inhibitory concentration (MIC) of vancomycin. **Methods**: an observational study conducted in a tertiary hospital, involving 62 clinical isolates of *S. aureus*. In addition, the MIC of vancomycin was determined using the Etest®, broth microdilution (BMD), and Vitek® automated system methods. Clinically relevant antimicrobial susceptibility profiles were evaluated, and agreement between methods was analyzed by statistical correlation using IBM-SPSS® software. The study was approved by the hospital's Research Ethics Committee under registration number 5.557.687/2022. **Results**: methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) isolates were susceptible to vancomycin, teicoplanin, linezolid, daptomycin, and tigecycline. A better correlation was observed between the vancomycin MIC values obtained by Vitek®2 and BMD compared to Etest®. However, Vitek®2 tended to overestimate MIC compared to BMD. **Conclusion**: the results indicate the importance of using complementary methods to validate high vancomycin MIC values, contributing to more appropriate therapeutic decisions and the prevention of bacterial resistance.

Keywords: bloodstream infection; *Staphylococcus aureus*; bacterial resistance; antimicrobials.

Resumo

Objetivo: avaliar o perfil de suscetibilidade antimicrobiana de isolados de *Staphylococcus aureus* provenientes de hemoculturas e comparar métodos para determinação da concentração inibitória mínima (CIM) da vancomicina. **Métodos**: estudo observacional realizado em um hospital terciário, envolvendo 62 isolados clínicos de *S. aureus*. Além disso, a determinação da CIM da vancomicina foi realizada pelos métodos Etest®, microdiluição em caldo (BMD) e sistema automatizado Vitek®. Os perfis de suscetibilidade a antimicrobianos de relevância clínica foram avaliados, e a concordância entre os métodos foi analisada por correlação estatística no software IBM-SPSS®.O estudo foi aprovado pelo Comitê de Ética em Pesquisa do hospital, sob o número de registro 5.557.687/2022. **Resultados**: isolados sensíveis à meticilina (MSSA) e resistentes à meticilina (MRSA) apresentaram suscetibilidade à vancomicina, teicoplanina, linezolida, daptomicina e tigeciclina. Observou-se melhor correlação entre os valores de CIM da vancomicina obtidos pelo Vitek®2 e pelo BMD em comparação ao Etest®. Porém, o Vitek®2 apresentou tendência à superestimação da CIM em relação ao BMD. **Conclusão**: os resultados indicam a importância da utilização de métodos complementares para validação de valores elevados de CIM da vancomicina, contribuindo para decisões terapêuticas mais adequadas e para a prevenção da resistência bacteriana.

Palavras chave: infecção da corrente sanguínea; *Staphylococcus aureus*; resistência bacteriana aos antimicrobianos.

INTRODUCTION

Staphylococcus aureus is one of the main etiological agents of bloodstream infections (BSI), with an annual incidence of approximately 50 cases per 100,000 inhabitants in developed countries, despite advances in antimicrobial therapy and infection control measures[1]. This bacterium is responsible for both community- and hospital-acquired infections and represents a major global concern due to the emergence of multidrug-resistant strains, including methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA), and vancomycin-resistant *S. aureus* (VRSA)[2,3].

The widespread use of antimicrobials has contributed to the selection of resistant strains. Penicillin resistance in *S. aureus*

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is commonly mediated by the production of β-lactamase, encoded by the blaZ gene, which is often located on plasmids within transposons. Resistance to vancomycin, a glycopeptide widely used to treat MRSA infections, is usually associated with the acquisition of the vanA operon, although exposure to subinhibitory antimicrobial concentrations may also induce transient transcriptomic changes[4]. Due to its genetic plasticity and ability to acquire resistance through mutations or mobile genetic elements, *S. aureus* exhibits a high capacity for antimicrobial resistance development[5,6]. Vancomycin resistance may arise from intrinsic mechanisms and/or transferable resistance gene cassettes[3].

Accurate and rapid methods for antimicrobial susceptibility testing are essential for appropriate clinical management. Automated systems such as Vitek®2 have been developed to identify microorganisms and determine susceptibility based on minimum inhibitory concentration (MIC), defined as the lowest antibiotic concentration capable of inhibiting bacterial growth[7,8]. However, several studies have reported inconsistent and variable vancomycin MIC results for *S. aureus* when using automated methods compared to broth microdilution (BMD), considered the gold standard by the Clinical and Laboratory Standards Institute (CLSI), and the gradient diffusion method (Etest®)[8].

Discrepancies among methods have been demonstrated in clinical studies. Kuo et al. reported that only 53.57% of vancomycin MIC values obtained by Vitek®2 were concordant with BMD results in MRSA isolates[8]. Additionally, Etest® frequently yields higher MIC values than BMD, which may negatively impact therapeutic decisions and clinical outcomes 9. In this context, the present study aims to compare the accuracy and reliability of different methods for determining vancomycin MIC in *S. aureus* isolates from blood cultures in a tertiary cardiopulmonary hospital in Brazil.

METHODS

Sample Collection

The cross-sectional analytical study with a quantitative approach was carried out with 62 *Staphylococcus aureus* isolates cultured from blood culture samples collected from patients at Hospital Dr. Carlos Alberto Studart Gomes (Ceará, Brazil) from August 2022 to July 2023. The hospital is a tertiary public institution specialized in the diagnosis and treatment of heart and lung diseases. It has 491 inpatient beds, including 70 Intensive Care Unit (ICU) beds, and performs highly complex procedures, such as adult and pediatric heart transplants. Only *S. aureus* isolates identified from blood cultures were included for the study, and those with polymicrobial growth were excluded. The study was approved by the hospital's research ethics committee under registration number 5,557,687/2022.

Data of gender, age, clinical outcome (hospital discharge or death), length of hospital stay, and hospital unit were too collected. Species identification was performed using the Gram-positive card of Vitek®2 (BioMèrieux, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was assessed by the automated method using suspension on the 0.5 McFarland scale on Vitek®2 637 antibiogram cards according to the 2019 CLSI standards. MIC was determined by broth microdilution assay, according to CLSI 10. Bacterial suspensions (1.5×106 CFU/mL) were used in 96-well plates in the presence of serial dilutions of the antimicrobial vancomycin (Sigma-Aldrich, product number V2002) (4 – 0.0625 µg/mL). Then, the plates were incubated at 37°C and visually inspected after 24 hours. The MIC was determined by the lowest concentration of the antibiotic capable of completely inhibiting bacterial growth, as evidenced by the absence of visible turbidity.

The determination of the MIC by the Etest® concentration gradient method (BioMèrieux, France) was performed using 0.5 McFarland scale suspensions, and seeded in sterile Mueller-Hinton agar. The Etest® strip with vancomycin (256 - 0.016 µg/mL) was centered on the plate. So, the plates were incubated at 37°C for 24 hours. Thereafter, the concentration at which the inhibitory halo intercepts the Etest® strip as the MIC was observed. Additionally, strains of *S. aureus* ATCC 6538p (MSSA) and ATCC 33591 (MRSA), donated by the Oswaldo Cruz Foundation (FIOCRUZ, Ceará, Brazil), were used as controls.

Statistical analysis

All experiments were performed in triplicate (n = 3). Statistical analysis was performed using IBM-SPSS® version 20 statistical software. Categorical variables had their distributions verified by the chi-square test. The normality of quantitative variables was determined by the Shapiro-Wilk test. For the parametric quantitative variables, the means were compared using the independent t-test or One-way analysis of variance (ANOVA). Pearson's correlation was used to evaluate the linear relationship between the variables. The Cohen Kappa test was used to analyze agreement and reliability. Statistical significance was reached at $P < 0.05$.

Ethics statement

The study was approved by the hospital's research ethics committee under registration number 5,557,687 on August 2nd, 2022.

RESULTS

General characteristics of patients

During the study period, the hospital had a total of 7,361 requests for blood cultures, with 1,299 (17.65%) testing

positive, and 62 (4.80%) positive samples for *S. aureus*. The general characteristics of the patients are shown in Table 1. Furthermore, there was no difference between the ages of patients infected with MRSA and MSSA strains (P = 0.731). After analyzing the hospital units, it showed a higher incidence of blood infections in inpatient units for both MRSA (74%) and MSSA (64.1%) (P = 0.0247). Among the isolates, 23 (37.10%) were classified as MRSA, while 39 (62.90%) were oxacillin susceptible (MSSA) (Table 1). Regarding the length of stay (LoS), there was an association between MRSA strains (around 51 days) and a longer LoS (P = 0.014) when compared to MSSA infections (around 32 days). A number of 22 patients died as a clinical outcome, with an equal proportion for MRSA and MSSA, thus showing no difference between the groups (P = 0.1188) (Table 1).

Table 1. General characteristics of patients with *S. aureus* (MRSA and MSSA) present in blood cultures, from August 2022 to July 2023.

	MRSA n=23	MSSA n=39	P-valor*
Gender (%)			
Male	13 (56.5%)	27 (69.2%)	0.0788 [a]
Female	10 (43.5%)	12 (30.8%)	
Age (years)	51.46±5.092	55.2±4.379	0.5860 [b]
Age range (years)			
0-10	3 (13%)	4 (10.2%)	0.7314 [a]
11-59	10 (43.5%)	14 (35.9%)	
>60 AM	10 (43.5%)	21 (53.9%)	
Outcome n (%)			
Discharge	12 (52.2%)	28 (71.8%)	0.1188 [a]
Death	11 (47.8%)	11 (28.2%)	
Length of hospital stay (days)	50.91±5.069	32.13±3.084	0.0014 [b]
Unit n (%)			
Emergency	3 (13%)	11 (28.2%)	0.0247 [a]
ICU	3 (13%)	3 (7.7%)	
Inpatient Units	17 (74%)	25 (64.1%)	

MRSA=Methicillin-resistant *Staphylococcus aureus*; MSSA=Methicillin-sensitive *Staphylococcus aureus*. *P < 0.05; a Chi-square; b Unpaired t-test.
Source: Falcão RM, et al., 2026.

Antimicrobial Susceptibility Profile of clinical isolates

In general, MRSA strains showed lower susceptibility rates. Benzylpenicillin was the drug with the highest resistance rates, reaching 0% susceptibility among MRSA. For the antimicrobials, levofloxacin and trimethoprim/sulfamethoxazole, the incidence of susceptibility was lower in the MRSA strains (Table 2).

Table 2. Susceptibility Profile of *S. aureus* MSSA and MRSA isolated from blood cultures through Vitek®2, from August 2022 to July 2023.

Antimicrobial	MRSA (n=23)	MSSA (n=39)
Benzylpenicillin	0 (0.00%)	5 (12.82%)
Oxacillin	0 (0.00%)	39 (100%)
Vancomycin	23 (100%)	39 (100%)
Gentamicin	22 (95.65%)	39 (100%)
Levofloxacin	17 (73.91%)	39 (100%)
Clindamycin	19 (82.61%)	25 (64.10%)
Linezolid	23 (100%)	39 (100%)
Daptomycin	23 (100%)	39 (100%)
Teicoplanin	22 (95.65%)	39 (100%)
Tigecycline	23 (100%)	39 (100%)
Rifampin	19 (82.61%)	37 (94.87%)
Trimethoprim/Sulfamethoxazole	17 (73.91%)	36 (92.31%)

MRSA=Methicillin-resistant *Staphylococcus aureus*; MSSA=Methicillin-sensitive *Staphylococcus aureus*.
Source: Falcão RM, et al., 2026

Evaluation of the Comparison of Methods for Determining the Minimum Inhibitory Concentration of Vancomycin

Vancomycin MIC was determined for the 62 isolates by three different methodologies (BMD, Vitek®2, and Etest®). From microdilution in broth and Vitek®2, MIC values between 0,5 and 2 µg/mL were obtained, and for the Etest® method, MIC values between 0.25 and 1 µg/mL were obtained. When comparing the results obtained by BMD and Vitek®2, divergence was identified in the MIC of 13 of the 62 isolates, with a variation of 1 dilution, obtaining an agreement of 79%. When comparing the MIC values of Etest® and Vitek®2, divergences were demonstrated in the MIC of 29 of the 62 isolates, with a variation of up to 2 dilutions, and the agreement attributed was 53.2%. And between Etest® and BMD, the agreement was 56.5%, with disagreements being found in 27 of the 62 isolates, with a variation of up to 2 dilutions. Cohen's Kappa test applied to vancomycin MIC obtained by these methods showed a weak reliability between Vitek®2 and BMD (K = 0.435; P < 0.001; agreement = 79%), with a confidence interval between 15% and 35%. There was considerable reliability between Vitek®2 and Etest® (K = 0.107; P > 0.05; agreement = 53.2%), with a confidence interval of 0% to 4%. The Kappa value for the crossing of Etest® and BMD was negative, where it was not possible to fit this result into the reliability parameters (K<0.01; P>0.05; agreement = 56.5%) (Table 3).

Table 3. Agreement between the Vitek®2, BMD and Etest® methods for determination of MIC (µg/mL) of vancomycin from *S. aureus* isolates (n=62).

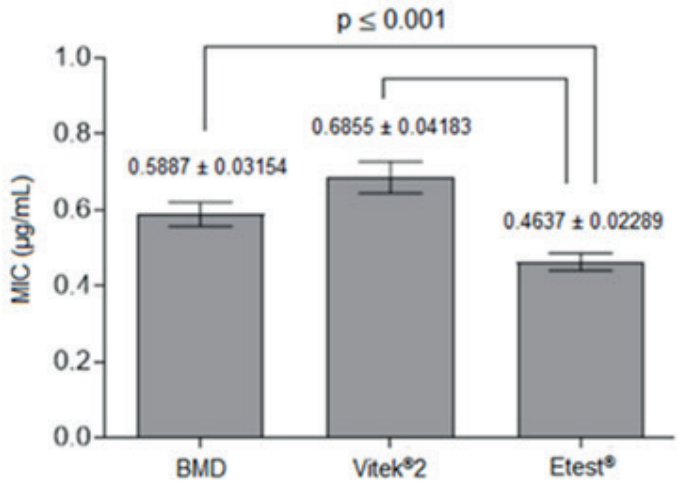
Staphylococcus aureus (CIM)	**STRAIN (ATCC)** **6538p (MSSA)** **33591 (MRSA)**	**Vitek®2** **0.5** **1.0**	**BMD** **0.5** **1.0**	**Etest®** **0.5** **0.5**
Vitek®2			BMD	
	0.5	1	2	Total
0.5	42 (67.7%)	1 (1.6%)	0 (0%)	43 (27.4%)
1	11 (17.7%)	6 (9,7%)	0 (0%)	17 (27,4%)
2	0 (0%)	1 (1.6%)	1 (1.6%)	2 (3,2%)
Total	53 (85.5%)	8 (12.9%)	1 (1.6%)	62 (100%)
			Agreement	79%
Vitek®2			Etest®	
	0.25	0,5	21	Total
0.25	13 (21%)	30 (48.4%)	0 (0%)	43 (69.4%)
0,5	4 (6.5%)	10 (16.1%)	3 (4,8%)	17 (27,4)
1	0 (0%)	1 (1.6%)	1 (1.6%)	2 (3.2%)
Total	17 (27.4%)	41 (66.1%)	4 (6.5%)	62 (100%)
		BMD	Agreement	53.2%
Etest®				
	0.5	1	2	Total
0.25	15 (24.2%)	2 (3.2%)	0 (0%)	17 (27.4%)
0.5	35 (56.5%)	6 (9.7%	0 (0%)	41 (66.1%)
1	3 (4.8%)	0 (0%)	1 (1.6%)	4 (6.5%)
Total	53 (85.5%)	8 (12.9%)	1 (1.6%)	62 (100%)
			Agreement	56.5%

BMD = Broth Microdilution; Etest®=Epsilometes test®.

Source: Falcão RM, et al., 2026

Regarding the geometric means of the MICs obtained between the methods, Vitek®2 presented a higher mean of the results (0.6855 ± 0.04183 µg/mL), followed by BMD (0.5887 ± 0.03154 µg/mL), and a lower mean was attributed to the Etest® (0.4637 ± 0.02289 µg/mL). Thus, there was a difference between the means obtained by Vitek®2 and Etest®, as well as between BMD and Etest® ($P < 0.01$) (Figure 1).

Figure 1. Geometric mean of vancomycin MIC of *S. aureus* isolates obtained by Vitek®2, BMD and Etest®. ANOVA ($P < 0.05$). BMD = Broth Microdilution; Etest® = Epsilometes test®; MIC= Minimum Inhibitory Concentration.



DISCUSSION

This study highlights the importance of bacterial surveillance in hospital settings to guide preventive and infection control measures and the appropriate use of antimicrobials. Analysis of *S. aureus* bloodstream infection revealed a predominance in male patients, corroborating findings of previous studies in Denmark[11]. Infection with the bacteria was found to be associated with advanced age, with a profile of infected patients with an average age of over 50 years. This finding was also shared by Escrihuela-Vidal et al. MRSA infection increases LoS similarly to previous studies, where LoS in MSSA infections represents around 70% of the LoS in MRSA infections 12,13. Our research found that ICUs had a lower incidence of cases (9.68%) due to the smaller number of beds available in the hospital for unknown reasons, despite its structural capacity for 70 beds. Previous studies corroborate the vulnerability of ICU patients to *S. aureus* bacteremia, highlighting factors such as invasive procedures (catheterization) and complex surgeries, the use of potent antimicrobials, and immunosuppressants, among others[14,15].

The data of the present study demonstrated that the rates of antimicrobial resistance observed for MRSA strains deserve to be highlighted since they presented a higher frequency and required attention. Antimicrobial resistance leads to a decrease in therapeutic options to treat *S. aureus* infections[10,16]. Although all strains have demonstrated susceptibility to vancomycin, a therapeutic option for MRSA infections, it is important to consider reports of emerging resistance to this antibiotic[17]. It is noteworthy that the phenomenon known as "MIC creep", in which MRSA strains may present increased MIC even though they are within the range of susceptibility to vancomycin, and thus may compromise the efficacy of antibiotic therapy, reinforces the need for precise methods to determine the antimicrobial susceptibility of these multidrug-resistant strains[1].

Furthermore, a phenomenon known as heteroresistance denotes the presence of subpopulations of bacterial cells with higher levels of antibiotic resistance than those of the rest of the population in the same culture, encompassing the occurrence of heterogeneous vancomycin-intermediate *S. aureus* (hVISA) and VISA. Heteroresistance can be detected by the population analysis profile (PAP) method, considered the gold standard. In this method, the bacterial population is subjected to a gradient of antibiotic concentrations on plates or in liquid medium, and bacterial growth at each of these concentrations is quantified more precisely. However, the typical PAP method is time-consuming and labor-intensive and may not be suitable for clinical laboratories that screen hundreds of isolates for heteroresistance[18].

Correct antimicrobial therapies and infection control measures together are of great importance for reducing the emergence of bacteria with high antibiotic resistance. The epidemiology of BSI caused by *S. aureus* may vary among localities or between countries, as demonstrated in a Canadian study[19]. In Brazil, MRSA strains have a relatively high incidence, and it is essential to consider not only their prevalence but also clinical findings as a result of their infections, such as the prolonged length of hospital stay observed in our study, which generates more burden for health systems 15,20. The isolation of MRSA strains from bacteremia is associated with a negative impact on the length of hospital stay, resulting in longer hospital stays, higher costs, and higher mortality rates[2,13].

The study also sought to evaluate the concordance of minimum inhibitory concentrations of vancomycin obtained by different methodologies. It was observed that the automated Vitek®2 method presented higher geometric means for vancomycin MIC compared to broth microdilution, and previous studies corroborate this trend, pointing out that Vitek®2 tends to overestimate vancomycin MIC[21]. Although all methodologies identified the isolates as susceptible to vancomycin, the concordance between Vitek®2 and BMD was higher than between Etest® and BMD, indicating a better correlation of Vitek®2 with BMD.

However, there were disagreements between the methods, with Etest® tending to underestimate the vancomycin MIC. The poor agreement between Etest® and BMD can be explained by the different MIC scales used to visualise the results, and the literature reports that Etest® tends to show reduced MIC values when the BMD MIC is less than or equal to 2 µg/mL[16]. These discrepancies are clinically relevant as they may influence the choice of treatment, leading to unnecessary administration of second-line antibiotics in patients with a vancomycin MIC considered elevated by Vitek®2 but not confirmed by BMD[16,22]. The precise determination of vancomycin MIC is complex and requires a careful approach in interpreting the results due to the inherent limitations of each method[23].

Therefore, due to the tendency to overestimate the MIC of Vitek®2, it is suggested that high MIC results for vancomycin, especially above 2 µg/mL, be associated with another method to confirm the result, in which case BMD would be an alternative. In case of discrepancy in results, the BMD result must be considered as it is the gold standard method according to CLSI 10,16. In addition, it is advisable to implement periodic quality controls using ATCC strains with known MICs to identify and correct possible flaws in the internal MIC determination process. These findings provide valuable insights for clinical practice and infection control policies, promoting a more effective approach to combating antimicrobial resistance.

Considering the implications of these findings for clinical practice, it is important to emphasize that the success of antimicrobial therapies depends not only on the search for new drugs but also on the choice of therapeutic protocol based on microbiological parameters such as reliable MIC assessment. To obtain a reliable MIC, many elements must be considered, such as the appropriate choice of method, adherence to labeling rules, and competent interpretation of results, in addition to pharmacokinetic/pharmacodynamic parameters.

The success of antimicrobial therapies depends not only on the search for new drugs, but also on the choice of therapeutic protocol based on microbiological parameters such as reliable MIC assessment. Information on the degree of susceptibility has great epidemiological and clinical value. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has greatly facilitated the assessment of the degree of susceptibility of microorganisms to antibiotics by introducing new criteria for interpreting results. These criteria distinguish between two levels of strain susceptibility. The first level concerns the standard dosage, while the second level refers to higher MIC values and requires longer exposure to the antibiotic, which would not be possible to obtain by approximate MIC values offered by automated systems such as VITEK or Phoenix[24].

CONCLUSION

The findings of this study highlight the importance of epidemiological knowledge of infections caused by multidrug-resistant strains, providing relevant data for the development of effective preventive strategies and treatments. A comparative analysis of vancomycin MIC determination revealed a better correlation between Vitek®2 and BMD results when compared to Etest®. However, it is critical to interpret the results of Vitek®2 with caution due to the identified tendency to overestimate MIC at a dilution. Therefore, it is suggested that the implementation of complementary methods to confirm elevated vancomycin MIC results by Vitek®2, being able to associate with the BMD gold standard methodology, for better diagnostic accuracy, avoiding the inappropriate use of antibiotics, and preventing the emergence of resistant strains.

Among the limitations of this study are the fact that the research was conducted at a single hospital, which may limit the generalizability of the findings to other hospitals that may exhibit different patterns of antimicrobial resistance; the relatively limited number of isolates, which, although sufficient to demonstrate significant differences between the methods for determining the MIC of vancomycin, could have been increased to improve the precision of the estimates and yield more robust analyses; and the absence of molecular characterization of the isolates, which could have facilitated an understanding of the mechanisms involved in resistance and the spread of strains of greater epidemiological relevance.

Furthermore, from a clinical perspective, although variables such as length of hospital stay, admission unit, and hospital outcome were evaluated, relevant therapeutic aspects, such as the antimicrobial regimens used, prior antibiotic exposure, the presence of invasive devices, or patient comorbidities, were not analyzed. Additionally, the comparison between the methods focused on the laboratory agreement of MIC values; the direct impact of the observed discrepancies on therapeutic decisions or patients' clinical outcomes was not assessed. This opens the door to prospective studies that integrate microbiological information and clinical data in order to more consistently clarify the relevance of these methodological differences in clinical practice.

However, despite these limitations, the results presented are relevant scientifically, clinically, and epidemiologically. The study provides updated data on the behavior of *Staphylococcus aureus* isolates causing bloodstream infections in a Brazilian tertiary care hospital, contributing to our understanding of the local epidemiology of antimicrobial resistance. Furthermore, it demonstrates in a practical way that different methodologies for determining the MIC of vancomycin can yield different results, highlighting the need for careful interpretation of susceptibility tests and the importance of using confirmatory methods in specific situations.

Therefore, these findings may contribute to the improvement of clinical microbiology practices, antimicrobial stewardship, and bacterial resistance surveillance programs, thereby supporting safer and more evidence-based therapeutic decisions. Given the global context of rising antimicrobial resistance, studies such as this one which provide evidence applicable to routine laboratory and hospital practice strengthen infection control strategies and yield benefits that extend beyond the academic setting, reaching patients, healthcare professionals, and healthcare systems.

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