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EVALUATION OF METHODS IN DETECTING VANCOMYCIN MIC AMONG MRSA ISOLATES AND THE CHANGES IN ACCURACY RELATED TO DIFFERENT MIC VALUES

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SUMMARY

INTRODUCTION: Methicillin-Resistant *Staphylococcus aureus* (MRSA) presenting reduced susceptibility to vancomycin has been associated to therapeutic failure. Some methods used by clinical laboratories may not be sufficiently accurate to detect this phenotype, compromising results and the outcome of the patient. **OBJECTIVES:** To evaluate the performance of methods in the detection of vancomycin MIC values among clinical isolates of MRSA. **MATERIAL AND METHODS:** The Vancomycin Minimal Inhibitory Concentration was determined for 75 MRSA isolates from inpatients of Mãe de Deus Hospital, Porto Alegre, Brazil. The broth microdilution (BM) was used as the gold-standard technique, as well as the following methods: E-test® strips (BioMérieux), M.I.C.E® strips (Oxoid), PROBAC® commercial panel and the automated system MicroScan® (Siemens). Besides, the agar screening test was carried out with 3 µg/mL of vancomycin. **RESULTS:** All isolates presented MIC ≤ 2 µg/mL for BM. E-test® had higher concordance (40%) in terms of global agreement with the gold standard, and there was not statistical difference among E-test® and broth microdilution results. PROBAC® panels presented MICs, in general, lower than the gold-standard panels (58.66% major errors), while M.I.C.E.® MICs were higher (67.99% minor errors). **CONCLUSIONS:** For the population of MRSA in question, E-test® presented the best performance, although with a heterogeneous accuracy, depending on MIC values.

KEYWORDS: Methicillin-Resistant *Staphylococcus aureus*; Vancomycin; Minimal Inhibitory Concentration.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important bacterial pathogens worldwide, especially in healthcare associated infections[7]. As MRSA is almost always multiresistant, vancomycin is the therapy of choice. In 2007, the Clinical and Laboratory Standards Institute (CLSI) determined the reduction of breakpoints for Minimal Inhibitory Concentration (MIC) of vancomycin among *S. aureus* to increase the sensitivity in detecting the non-susceptible isolates[5]. The apparent increase in vancomycin MIC among MRSA, observed in the last years, could represent the first step for the occurrence of fully resistant isolates. Indeed, the emergence of strains has been determined by presenting intermediate resistance (VISA) or hetero-VISA (vancomycin-intermediate *S. aureus*). Besides, increasing proportions of MRSA isolates with high MICs have been observed within the susceptible range, a phenomenon known as vancomycin MIC creep[8,10]. These isolates with MIC creep have been associated with therapeutic failure[13,18], as vancomycin may be ineffective against isolates with MICs between 1 and 2 µg/mL[8].

Several methods with variable sensitivity and specificity are available to determine vancomycin MIC. According to CLSI, broth microdilution (BM) is considered the gold standard[5]. However, because it is time-consuming, a considerable number of clinical laboratories do not use it as routine methodology. Other techniques have been widely used, with variable sensitivity and specificity, such as automated systems, strips with antimicrobial concentration gradient and microdilution commercial panels[6]. The objective of this study was to evaluate the accuracy of several methods in the characterization of vancomycin MIC among clinical MRSA isolates.

MATERIAL AND METHODS

Bacterial isolates: Seventy-five MRSA from Mãe de Deus hospital, a 400-bed general hospital in Porto Alegre, were evaluated in Southern Brazil. Methicillin resistance was first characterized by automated system (MicroScan Walk Away, Siemens®), MRSA phenotype was confirmed by molecular methods (*mec*A gene), described elsewhere[20]. Isolates were maintained (-20 ºC) in 10% Skim Milk (Difco, Detroit, USA) with 10% glycerol.

Determination of Minimal Inhibitory Concentration: Vancomycin

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MICs were determined by BM[6] and by the following techniques, according to the manufacturer's instructions: E-test® strips (BioMérieux, Marcy l'Étoile, France), M.I.C.E.® strips (Oxoid, Thermo Fisher Scientific, Basingstoke, UK), MicroScan and commercial panels for MIC detection (PROBAC®). Besides, the agar dilution screening test was performed with 3 µg/mL of vancomycin, as proposed by BURNHAM, WEBER & DUNNE[1]. A vancomycin-susceptible strain (ATCC 25923) and a positive control (*Enterococcus faecalis* carrying *van*A gene) were used for all methodologies.

Statistical analysis: Descriptive statistics were applied, and data were evaluated by ANOVA, followed by the Tukey *post hoc* test. The results were processed using the *Statistical Package for Social Sciences* (SPSS) 17.0. Results statistically significant were considered when $p < 0.05$.

RESULTS

The 75 MRSA evaluated were susceptible to vancomycin with MICs ≤ 2 µg/mL (BM): 2 µg/mL (4%), 1 µg/mL (50.66%), 0.5 µg/mL (42.66%), and 0.25 µg/mL (2.66%). The MIC_{50} and MIC_{90} were both 1 µg/mL. All isolates were susceptible to vancomycin for agar dilution screening. The MicroScan panel used had four dilution points (16, 8, 4 and 2 µg/mL) and all isolates presented MICs ≤ 2 µg/mL.

The E-test® was statistically similar to BM ($p = 0.777$). However, PROBAC® and M.I.C.E.® were both statistically different compared to the gold standard ($p < 0.001$).

For the E-test® analysis, two approaches were used: the first one used the gross values; for the second approach, CLSI breakpoints for BM were used to evaluate the E-test® (i.e. an E-test MIC of 3 was, for this approach, considered 4 µg/mL). This data are shown in Table 1.

The agreement among evaluated methods and BM was also evaluated, considering each MIC value to observe if the performance of the methods depended on the MIC value, as shown in Table 2.

Considerable heterogeneous performance was observed in different MIC values. In MRSA isolates with a vancomycin MIC of 0.25 µg/mL, the E-test® and M.I.C.E.® presented values at least 1-fold higher than BM for all isolates; which was also observed in most isolates with a MIC of 0.5 µg/mL. However, for a MIC of 1 and 2 µg/mL, higher agreements for both strip-based methodologies (Table 2) were observed. For these methods, discordant results showed MICs 1-fold higher than BM for MIC 1 µg/mL. On the other hand, for MIC 2 µg/mL, all discordant results presented MICs lower than the gold standard method. Regarding the commercial panel PROBAC®, better performances were observed in lower MICs (0.25 and 0.5 µg/mL). For MICs 1 and 2 µg/mL, discordance was a major concern (Table 2).

In terms of global agreement with the gold standard, the E-test® had higher concordance (40%) and it was the only one statistically similar to BM, followed by PROBAC® (36%), which had a higher number of major errors (58.66%). Minor errors were mostly observed for M.I.C.E.® strips (67.99%).

DISCUSSION

The therapeutic failure related to vancomycin is well established, especially regarding to MIC creeps[13]. Most hospitals report estimated vancomycin MICs through automated methods. However, different authors show evidence that MIC creeps are not accurately detected by automated systems[8,9]. The failure during vancomycin therapy is particularly associated to pharmacokinetic and pharmacodinamic characteristics of the drug, which needs a ratio area under the curve/MIC higher than 400 to obtain therapeutic success. When isolates present a MIC of 2 µg/mL, this ratio is hard to achieve, once serum vancomycin concentration should be 15 and 20 µg/mL[19].

In this study, the E-test® was, in general, the method with a higher agreement with BM, presenting the most homogeneous performance in different MIC values. The commercial panel PROBAC® presented better performance in lower MIC data regarding these panels, which is extremely relevant, considering the absence of previous information on

Table 1
Distribution of MRSA according to MIC values and methodologies

MIC (µg/mL)	Methodology % (*n*)				
	Broth microdilution	PROBAC®	E-test®	E-test®***	M.I.C.E.®
0.25	2.66% (2)	18.66% (14)	1.33% (1)	1.33% (1)	1.33% (1)
0.38*	NA**	NA**	5.33% (4)	NA**	NA**
0.50	42.66% (32)	68% (51)	14.66% (11)	20% (15)	2.66% (2)
0.75*	NA**	NA**	25.33% (19)	NA**	NA**
1.00	50.66% (38)	13.33% (10)	30.66% (23)	56% (42)	38.66% (29)
1.50*	NA**	NA**	22.66% (17)	NA**	NA**
2.00	4% (3)	0% (0)	0% (0)	22.66% (17)	57.33% (43)
	100% (75)	100% (75)	100% (75)	100% (75)	100% (75)

MRSA: Methicillin-resistant *Staphylococcus aureus*; MIC: Minimal Inhibitory Concentration; *MICs value observed only on E-test® strip; **NA = not applicable; ***CLSI breakpoints for BM were used for the evaluation of E-tests® results.

Table 2
Agreement (%) among methods and BM, according to vancomycin MIC values

Broth microdilution MIC (µg/mL)	Methodology	Agreement % (*n*)	Lower MIC[#] % (*n*)	1X higher[##] MIC % (*n*)	2X higher[###] MIC % (*n*)
0.25	PROBAC®	50.00% (1)	0.00% (0)	50.00% (1)	0.00% (0)
	E-test® *	0.00% (0)	0.00% (0)	50.00% (1)	50.00% (1)
	M.I.C.E.®	0.00% (0)	0.00% (0)	0.00% (0)	100.00% (2)
0.50	PROBAC®	59.37% (19)	31.25% (10)	9.38% (3)	0.00% (0)
	E-test® *	25.00% (8)	0.00% (0)	56.25% (18)	18.75% (6)
	M.I.C.E.®	3.12% (1)	3.12% (1)	28.13% (9)	65.63% (21)
1.00	PROBAC®	18.42% (7)	81.58% (31)	0.00% (0)	0.00% (0)
	E-test® *	55.26% (21)	18.42% (7)	26.32% (10)	0.00% (0)
	M.I.C.E.®	47.37% (18)	2.63% (1)	50.00% (19)	0.00% (0)
2.00	PROBAC®	0.00% (0)	100.00% (3)	0.00% (0)	0.00% (0)
	E-test® *	33.33% (1)	66.67% (2)	0.00% (0)	0.00% (0)
	M.I.C.E.®	66.67% (2)	33.33% (1)	0.00% (0)	0.00% (0)
Global agreement	PROBAC®	36.00% (27)	58.66% (44)	5.33% (4)	0.00% (0)
	E-test® *	40.00% (30)	12.00% (9)	38.66% (29)	9.33% (7)
	M.I.C.E.®	28.00% (21)	4.00% (3)	37.33% (28)	30.66% (23)

*CLSI breakpoints for BM were used for the evaluation of E-tests® results; [#]MICs were defined as lower than the BM; [##] MICs were defined as one-fold dilution higher than the BM; [###] MICs were defined as two-fold dilution higher than the BM.

the performance of this method. To the authors' knowledge, this is the first study to evaluate the accuracy of MICs determined by PROBAC® panels.

On the other hand, M.I.C.E.® had better performance with higher MICs. Global agreement of M.I.C.E.® (28%) was considerably lower than that observed by CAMPANA *et al.* (2011) (76.3%). Besides, MUSHTAQ *et al*. (2010) observed elevated rates of agreement between the strips (M.I.C.E.® and E-test®), concluding that both are appropriate for clinical laboratory use. In this study, the low global agreement of M.I.C.E.® strips does not point them as accurate methods.

VAN HAL *et al*. (2012), in his meta-analysis, showed no statistical difference between mortality associated with infections caused by *S. aureus* strains and vancomycin MIC of 1.5 µg/mL and 1 µg/mL. However, mortality associated with strains presenting MIC 2 µg/mL and 1.5 µg/mL was statistically different. Therefore, the interpretation of M.I.C.E.® results is compromised, once it does not present the 1.5 µg/mL value of MIC. So, the M.I.C.E.® MIC of 2 µg/mL may, in fact, represent 1.5 µg/mL or 2 µg/mL, which could lead to therapeutic failure.

According to SWENSON *et al*. (2009) and RYBAC *et al.* (2013), the E-test® and MicroScan lead to a higher BM of MIC 1-fold. CDC recommends that the clinical laboratory should define an algorithm to determine which additional tests would be necessary to confirm an *S. aureus* as having reduced susceptibility to vancomycin. This algorithm should consider characteristics of patients and resources available in the clinical laboratory[3]. As MIC average of population may affect performance of tests, it should be considered when choosing alternative methodologies for broth microdilution.

For the MRSA isolates tested, the E-test® presented the best performance. Even though, overestimated MIC, also described by other authors, compromises the accuracy of the method. Nevertheless, these non-accurate MICs represent minor errors, which have lower impact on the treatment of patients, compared to major errors. So, this data support the use of the E-test® as a rapid and easy test.

This study has some limitations. First, the reduced number of isolates could have compromised the statistical analysis. Second, the MRSA population tested presented low MICs; studies with a different population of MRSA must be conducted to evaluate the performance of methods in strains with higher chances of leading to therapeutic failures and determining if differences in performance would also be observed.

Another point of concern is that MIC values may suffer alterations after cryopreservation. EDWARDS *et al.* (2012) demonstrated that MICs from automated systems and the E-test® were significantly lower after cryopreservation, if compared with those from the E-test® analysis, at the time of isolation, either for vancomycin and daptomycin. SCHAUMBURG *et al.* (2014) also pointed out that the prevalence of vancomycin MIC creeps may be underestimated because of the cryopreservation effect. Therefore, vancomycin MIC creeps might be lost after cryoconservation[8,16]. This variable was not considered as the study population. Further studies must be designed to reinforce previous observations.

Monitoring the occurrence of *S. aureus* with reduced susceptibility to vancomycin is a subject. For the population of MRSA tested, the E-test® presented the best performance, although with heterogeneous accuracy,

depending on MIC values. Thus, the choice of method to determine MIC values must take into consideration costs, conditions of the clinical laboratory and the characteristics of the *S. aureus* populations evaluated.

RESUMO

Avaliação de métodos na detecção da MIC de vancomicina e mudanças na acurácia relacionada a diferentes valores de MIC

INTRODUÇÃO: *Staphylococcus aureus* resistente à meticilina (MRSA) apresentando suscetibilidade reduzida à vancomicina tem sido associado à falha terapêutica. Alguns métodos utilizados por laboratórios clínicos podem não ser suficientemente precisos para detectar este fenótipo, comprometendo os resultados e o desfecho do paciente. **OBJETIVOS:** Avaliar o desempenho de métodos na detecção dos valores de MIC de vancomicina entre isolados clínicos de MRSA. **MATERIAIS E MÉTODOS:** Determinamos a Concentração Inibitória Mínima de Vancomicina para 75 MRSA isolados de pacientes do Hospital Mãe de Deus, Porto Alegre, Brasil. Utilizamos a microdiluição em caldo como técnica padrão-ouro e os seguintes métodos: tiras de E-test® (BioMérieux), tiras M.I.C.E® (Oxoid), painel comercial da PROBAC® e sistema automatizado MicroScan® (Siemens). Além disso, foi realizado o teste de triagem em ágar com 3 µg/mL de vancomicina. **RESULTADOS:** Todos os isolados apresentaram MIC ≤ 2 µg/mL. Não houve diferença estatística entre os resultados do E-test® e da microdiluição em caldo. O painel da PROBAC® apresentou MICs, em geral, menores que o padrão-ouro (58,66% de erros maiores), enquanto que as MICs pelo M.I.C.E.® foram maiores (67,99% de erros menores). **CONCLUSÕES:** Para nossa população de MRSA, E-test® apresentou o melhor desempenho, embora com uma acurácia heterogênea, dependendo dos valores da MIC.

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