Letter to the Editor

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

Aline GP Sousa[1], Thaina M da Costa[1], Fernanda S Cavalvante[1], Raiane C Chamon[1], Dennis C Ferreira[1,3], Simone A Nouér[2], Kátia RN dos Santos[1]

[1] *Departamento de Microbiologia Médica, Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*
[2] *Hospital Universitário Clementino Fraga Filho and Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*
[3] *Department of Oral Medicine, School of Dentistry, Veiga de Almeida University, Rio de Janeiro, Brazil*

Key words: Bloodstream infections; *Staphylococcus aureus*; susceptibility methods; vancomycin

J Infect Dev Ctries 2014; 8(4):558-560. doi:10.3855/jidc.4395

(Received 07 November 2013 – Accepted 08 January 2014)

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 $\log_2$ concentration) higher than those tested by the BMD method, whereas the Etest method showed 37 (29.8%) isolates with MIC values four times (2 $\log_2$ 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

Table 1. Vancomycin MICs determined by three different methods in 124 *Staphylococcus aureus* isolates from bloodstream infections

Method	No (%) of results with vancomycin MIC (µg/ml) of:				
	0.5	1.0	1.5	2.0	3.0
BMD[a]	77 (62%)	47(38%)	0	0	0
AD[b]	6 (4.8%)	116 (93.5%)	1 (0.8%)	1 (0.8%)	0
Etest	0	54 (44%)	53 (43%)	15 (12%)	2 (1.6%)

[a] BMD: broth microdilution; [b]AD: agar dilution.

Table 2. Dilution difference of two test methods compared with the results obtained by the broth microdilution reference method

Method	No. of results with dilution difference of [a]:					% of results with dilution difference of ±1[b]:
	-2	-1	0	+1	+2	
AD[c]	0	0	51	73	0	100
Etest	0	0	12	75	37	70

[a] -2: result by the test method is 2 $\log_2$ concentrations lower than the Broth microdilution (BMD) MIC; -1: result by the test method is 1 $\log_2$ 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 $\log_2$ concentration greater than the BMD MIC; +2: result by the test method is 2 $\log_2$ concentrations greater than the BMD MIC.; [b] Essential agreement; [c]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.

Acknowledgements

This study was supported by grants from: Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), Fundação Universitária José Bonifácio (FUJB) and Programa de Núcleos de Excelência (PRONEX).

References

1. Marra AR, Camargo LF, Pignatari AC, Sukiennik T, Behar PR, Medeiros EA, Ribeiro J, Girão E, Correa L, Guerra C, Brites C, Pereira CA, Carneiro I, Reis M, de Souza MA, Tranchesi R, Barata CU, Edmond MB, Brazilian scope study group 2011 (2011) Nosocomial bloodstream infections in Brazilian hospitals: analysis of 2,563 cases from a prospective nationwide surveillance study. J Clin Microbiol 49: 1866-1871.
2. Levine DP (2006) Vancomycin: a history. Clin Infect Dis 42: S5-S12.
3. Kruzel MC, Lewis CT, Welsh KJ, Dundas NE, Mohr JF, Armitige LY, Wanger A (2011) Determination of vancomycin and daptomycin MICs by different testing methods for methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 49: 2272-2273.
4. Dhand A and Sakoulas G (2012) Reduced vancomycin susceptibility among clinical *Staphylococcus aureus* isolates ('the MIC Creep'): implications for therapy. Med Rep 4: 4.
5. Bannerman TL and Peacock SJ (2007) *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci. In Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC. 390–411
6. Burnham CA, Weber CJ, Dunne WMJr (2010) Novel screening agar for detection of vancomycin-nonsusceptible *Staphylococcus aureus.* J Clin Microbiol 48: 949-951.
7. Chen X, Wang WK, Han LZ, Liu Y, Zhang H, Tang J, Liu QZ, Huangfu YC, Ni YX (2013) Epidemiological and genetic diversity of *Staphylococcus aureus* causing bloodstream infection in Shanghai, 2009-2011. PLoS One 8: e72811.
8. Eshwara VK, Munim F, Tellapragada C, Kamath A, Varma M,Lewis LE, Mukhopadhyay, C (2013) *Staphylococcus aureu*s bacteremia in an Indian tertiary care hospital: observational study on clinical epidemiology, resistance characteristics, and carriage of the Panton-Valentine leukocidin gene. Int J Infect Dis 17: e1051-1055
9. Gould FK, Brindle R, Chadwick PR, Fraise AP, Hill S, Nathwani D, Ridgway GL, Spry MJ, Warren RE (2009) Guidelines (2008) for the prophylaxis and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the United Kingdom. J Antimicrob Chemother 63: 849-861.
10. Mazzariol A, LoCascio G, Kocsis E, Maccacaro L, Fontana R, Cornaglia G (2012) Outbreak of linezolid-resistance *Staphylococcus haemolyticus* in an Italian intensive care unit. Eur J Clin Microbiol Infect Dis 31: 523-527.
11. Gales AC, Sader HS, Andrade SS, Lutz L, Machado A, Barth A (2006) Emergence of linezolid-resistant *Staphylococcus aureus* during treatment of pulmonary infection in a patient with cystic fibrosis. Int J Antimicrob Agents 27: 300-302.
12. Prakash V, Lewis JS, Jorgensen JH (2008) Vancomycin MICs for methicillin-resistant *Staphylococcus aureus* isolates differ based upon the susceptibility test method used. Antimicrob Agents Chemother 52: 4528.
13. Rybak MJ, Vidaillac C, Sader HS, Rhomberg PR, Salimnia H, Briski LE, Wanger A, Jones RN (2013) Evaluation of vancomycin susceptibility testing for methicillin-resistant *Staphylococcus aureus*: comparison of Etest and three automated testing methods. J Clin Microbiol 51: 2077-2081.
14. Swenson JM, Anderson KF, Lonsway DR, Thompson A, Mcallister SK, Limbago BM, Carey RB, Tenover FC, Patel JB (2009) Accuracy of commercial and reference susceptibility testing methods for detecting vancomycin-intermediate *Staphylococcus aureus*. J Clin Microbiol 47: 2013-2017.
15. Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, Stellrecht K (2008) Relationship between vancomycin MIC and failure among patients with methicillin-resistant *Staphylococcus aureus* bacteremia treated with vancomycin. Antimicrob Agents Chemother 52: 3315-3320.

Corresponding author

Kátia RN dos Santos
Laboratório de Infecção Hospitalar, Departamento de Microbiologia Médica, Instituto de Microbiologia Paulo de Góes, CCS, Bloco I, Sala 010, UFRJ.
Cidade Universitária, Rio de Janeiro, RJ, Brazil.
CEP: 21941-590.
Phone: 55-21-2560-8344,
Fax: 55-21-2560-8028.
E-mail: santoskrn@micro.ufrj.br.

Conflict of interests: No conflict of interests is declared.