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Short Communication

Erroneous Reporting of Vancomycin Susceptibility for *Staphylococcus* spp. by Vitek Software Version 2.01

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SUMMARY: The reporting of vancomycin resistance in *Staphylococcus* spp. has enormous therapeutic and epidemiological consequences. We evaluated Vitek software version 2.01 by the CLSI-recommended broth dilution method as well as the CDC-recommended vancomycin screen agar and Etest for vancomycin susceptibility testing of *Staphylococcus* spp. Of the 105 isolates of *Staphylococci* tested by the above methods, Vitek 2.01 gave 16 (15%) a false vancomycin intermediate/resistant phenotype. Laboratories using automated systems for routine microbiological susceptibility testing must confirm such resistance by validated methods.

The detection of *Staphylococci* with reduced susceptibility to vancomycin is an important issue for clinical microbiology laboratories. The Vitek identification and susceptibility system (BioMérieux, Marcy l'Etoile, France) is one of the frequently used automated methods in clinical microbiology laboratories to detect decreased susceptibility to vancomycin. Earlier reports indicated that the previous versions of Vitek reported vancomycin MICs in the Clinical and Laboratory Standards Institute (CLSI) susceptible range (≤0.5 - 4 μg/ml) in isolates for which the vancomycin MICs were actually in the intermediate range (8 - 16 μg/ml) (1). In another report, Vitek version 7.01 had improved sensitivity for detecting *Staphylococci* with reduced susceptibility to vancomycin than earlier software versions, although an overall upward shift in vancomycin MIC was noted; however, for the most part, this did not affect the categorical interpretations of the results (2). BioMérieux recently introduced version 2.01 that is compatible with Vitek 2 compact instruments (3). This new version, introduced in the first quarter of 2007 and evaluated in the present study, has received US Food and Drug Administration (FDA) clearance for vancomycin-resistant *Staphylococcus aureus* (VRSA) screening. VRSA screening predicts the presence of high levels of vancomycin resistance, meaning those strains of *S. aureus* with an MIC of >16 μg/ml.

According to the current CLSI guidelines, *Staphylococci* for which the vancomycin MIC is ≤2 μg/ml are considered susceptible, while those having MIC of 4 - 8 μg/ml are considered intermediate and those with MIC ≥16 μg/ml are resistant (3). We compared the vancomycin susceptibility of 105 consecutive isolates of *S. aureus* and coagulase-negative *Staphylococci* (CoNS) by Vitek 2.01 using AST GP 61 cards and the CLSI-recommended broth microdilution method (4), vancomycin screen agar, and Etest methods (4).

The study was conducted in two parts. In the first part, 65 consecutive isolates of *S. aureus* and 40 isolates of various species of CoNS (19 *S. haemolyticus*, 9 *S. epidermidis*, 7 *S. hominis*, and 1 each of *S. lugdunensis*, *S. xylosus*, *S. intermedius*, *S. capitis*, and *S. chromogenes*) were included. Of these, 49 (75%) isolates of *S. aureus* and all CoNS were methicillin resistant by the cefoxitin (30 μg) and oxacillin (1 μg) disk methods (5) as well as by the Vitek 2 system. All 105 isolates of *Staphylococci* were tested for vancomycin susceptibility by the disk diffusion method according to the CLSI guidelines (3), the vancomycin screen agar method (performed according to Centers for Disease, Control and Prevention [CDC] guidelines) (4), and by the Vitek 2 compact system 2.01. The *S. aureus* ATCC 25923, *S. aureus* ATCC 700699 (vancomycin-intermediate *S. aureus* [VISA]), *S. aureus* ATCC 43300, *S. epidermidis* ATCC 12228, and *Enterococcus faecalis* 51299 strains were used as controls for all sensitivity testing methods. The inoculum for Vitek 2 was prepared according to the manufacturer's instructions. Briefly, a McFarland 0.5 standard solution was prepared in 0.45% sterile sodium chloride solution, provided by the manufacturer.

All the isolates had a vancomycin zone diameter ≥15 mm, and none of them showed any growth on vancomycin screen agar plates. However, two isolates of *S. aureus* and seven isolates of CoNS had vancomycin MICs in the intermediate range (8 μg/ml); one *S. aureus* and four isolates of CoNS had vancomycin MICs of 16 μg/ml, and two isolates of CoNS had vancomycin MIC ≥32 μg/ml according to the Vitek 2 system. Of the CoNS showing a vancomycin-intermediate phenotype by the Vitek 2 system, four were *S. haemolyticus*, two were *S. hominis*, and one was *S. intermedius*. Of the CoNS showing a vancomycin-resistant phenotype by the Vitek 2 system, three were *S. hominis* and one each was *S. capitis*, *S. epidermidis*, and *S haemolyticus*.

In the second part of the study, all 16 isolates showing vancomycin MICs in the intermediate or resistant range according to the Vitek 2 system were retested with Vitek 2. The MICs of vancomycin for these isolates were also determined by the broth microdilution method (performed according to the CLSI guidelines) (3) and the vancomycin Etest, performed according to the manufacturer's instructions (AB Bio Disk, Solna, Sweden). The *S. aureus* ATCC 25923, *S. aureus* ATCC 700699 (VISA), *S. aureus* ATCC 43300, *S. epidermidis* ATCC 12228, and *E. faecalis* 51299 strains were used as controls. Control strains (*S. aureus* ATCC 25923, *S. aureus* ATCC 43300, *S. epidermidis* ATCC 12228, and *E. faecalis* 51299) had vancomycin MICs in the susceptible range (<2 μg/ml)

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Table 1. Comparison of vancomycin susceptibility test results of *Staphylococci* judged to be intermediate or resistant by the Vitek system

Organism $n = 16$	Minimum inhibitory concentration in μg/ml (n)		
	Vitek 2	Broth microdilution	Etest
CoNS ($n = 2$)[1)]	≥32	0.5 (2)	0.38 (2)
S. aureus ($n = 1$)	16	0.25 (2)	0.125 (2), 0.38 (1),
CoNS ($n = 4$)[2)]		0.5 (3)	0.25 (2)
S. aureus ($n = 2$)	8	0.5 (7)	0.38 (6)
CoNS ($n = 7$)[3)]		0.125 (2)	0.25 (2)
			0.125 (1)

[1)]: *S. hominis* (1), *S. haemolyticus* (1).
[2)]: *S. hominis* (2), *S. epidermidis* (1), *S. capitis* (1).
[3)]: *S. haemolyticus* (4), *S. hominis* (2), *S. intermedius* (1).
≥16 μg/ml, resistant; 4 - 8 μg/ml, intermediate; ≤ 2 μg/ml, sensitive.
n, no. of isolates; CoNS, coagulase-negative *Staphylococci*.

by broth dilution, the Etest, and vancomycin screen agar. *S. aureus* ATCC 700699 (VISA) had vancomycin MIC 6 μg/ml by broth dilution, the Etest and vancomycin screen agar. The vancomycin MIC of *S. aureus* ATCC 700699 (VISA) was 4 μg/ml according to Vitek 2.The final reading of the Etest was taken after 24 h (4). Although the Vitek MICs were found to be reproducible, all the isolates were susceptible (<2 μg/ml) by the broth dilution and Etest methods. The vancomycin MICs of these 16 isolates by the various methods are shown in Table 1.

Vancomycin resistance in *S. aureus* is difficult to define, mainly because of methodological problems in their detection (6). In the absence of vancomycin pressure, vancomycin resistance is unstable and is expressed at a low level. This low-level expression of vancomycin resistance in *S. aureus* may be the reason why these strains are difficult to detect clinically. The CLSI disk diffusion method of sensitivity by standard 30 μg vancomycin frequently misclassifies intermediately susceptible isolates as fully susceptible. Presently, MIC determinations by broth or agar dilution are the gold standard for assessing vancomycin susceptibility, but these methods are not suitable for routine use in diagnostic laboratories (6). Both conventional and automated methods have problems in distinguishing VISA isolates from vancomycin-susceptible strains. Vancomycin resistance not only has enormous therapeutic implications, but is important also from epidemiological and infection control standpoints (6). Therefore, every institution must carefully evaluate the screening methods being used for vancomycin resistance in order to correctly report sensitivity against this important antimicrobial. Laboratories that depend exclusively on automated sensitivity testing methods must regularly validate their systems. We recommend that isolates showing high vancomycin MICs screened by Vitek 2 alone should be retested using an alternative CLSI-approved method, along with control strains, to confirm phenotype resistance.

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