

COMPARISON OF BROTH MICRODILUTION AND ETEST METHOD RESULTS WHEN TESTING VANCOMYCIN AND DAPTOMYCIN AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM 9 USA HOSPITALS

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ABSTRACT

Background: Increased vancomycin MIC values within the CLSI susceptibility range (1.5-2 µg/ml), have been correlated to poor clinical outcome, forcing clinical laboratories to assess exact MICs by reference or alternative methods, such as Etest (AB BIODISK). We evaluated the correlation between vancomycin and daptomycin MIC values generated by Etest and CLSI broth microdilution (BMD).

Methods: Randomly selected methicillin-resistant *Staphylococcus aureus* (MRSA) blood-stream strains were collected from 9 USA hospitals (1 per CDC census region) from 2002-2006 (40 strains/year/center). Vancomycin and daptomycin MIC results were determined by BMD using frozen-form panels with incremental dilutions (example: 20 dilutions between 1.5 and 0.25 µg/ml) and by Etest.

Results: 1800 strains (200/hospital) were evaluated. Modal MIC values for BMD and Etest were 0.625 (41.6% of values) and 1.5 µg/ml (58.2%) for vancomycin; and 0.156 (31.6%) and 0.375 µg/ml (37.2%) for daptomycin, respectively. Compared to BMD, Etest MIC results were 1-1.5 and 0.5-1 doubling dilutions higher for vancomycin and daptomycin, respectively (see Table 1). This resulted in a shift of vancomycin MIC mode to 2 µg/ml and an additional 1,606 strains (89.2%) would be labeled at-risk for clinical failure; whereas the true MIC mode was only 0.625 µg/ml.

Conclusions: Etest provides vancomycin and daptomycin MICs consistently higher (0.5-1.5 log₂ dilutions) than BMD. Susceptibility method used can have a great impact on physician decisions for vancomycin and daptomycin treatment. Dominant population (91.2% of MRSA) would be categorized as vancomycin non-susceptible by Etest method if the susceptible breakpoint was adjusted to ≤1 µg/ml, as suggested by clinical outcome data. Daptomycin was slightly less affected (+0.5-1 dilutions and 0.4% false non-susceptible); microbiologist should use Etest with caution.

INTRODUCTION

Vancomycin is still extensively used for treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia as well as other MRSA infections. However, vancomycin treatment failure is not uncommon, even when MRSA are fully susceptible to vancomycin by criteria used by the Clinical and Laboratory Standards Institute (CLSI; breakpoint MIC, ≤2 µg/ml). A reduction in the efficacy of vancomycin against MRSA strains with elevated vancomycin MIC (1-2 µg/ml) has been widely reported, suggesting that slight elevations in the MIC may explain suboptimal clinical outcome.

The majority of clinical laboratories use automated systems or disk diffusion method as the routine susceptibility testing technique. However, the disk diffusion method and some automated systems do not accurately detect vancomycin-intermediate *S. aureus* (VISA). In addition, there has been a growing number of reports showing discrepancies between in vitro susceptibility test results for vancomycin and clinical outcome of MRSA infections treated with this antimicrobial. Thus, many laboratories are being requested to assess exact MIC values by reference or alternative methods, such as Etest® (AB BIODISK, Solna Sweden).

In the present study, we evaluated the correlation between vancomycin and daptomycin MIC values generated by Etest and CLSI broth microdilution methods.

METHODS

Bacterial Isolates: Nine hospitals, one from each of the United States census regions were selected to participate in the study. Each medical center contributed 200 MRSA strains from bloodstream infections collected from 2002 through 2006 (target, 40 per year).

Participant centers:

1. New England Medical Center, Boston, MA.
2. New York Hospital Queens, New York, NY.
3. Ochsner Clinic Foundation, New Orleans, LA.
4. University of Colorado Health Science Center, Denver, CO.
5. University of Nebraska Medical Center, Omaha, NE.
6. University of Washington, Seattle, WA.
7. University of Alabama at Birmingham, Birmingham, AL.
8. Wayne State University, Detroit, MI.
9. Medical University of South Carolina, Charleston, SC.

Susceptibility testing: Minimum inhibitory concentration (MIC) values were determined by reference broth microdilution method (frozen-form panels) for daptomycin, vancomycin and oxacillin with appropriate medium variations (50 mg/L of calcium) for testing daptomycin. Thirty six dilutions between 64 and 0.06 µg/ml were tested for vancomycin and 20 dilutions between 16 and 0.06 µg/ml were tested for daptomycin. Isolates were also susceptibility tested against daptomycin and vancomycin by Etest, according to manufacturer recommendations (AB BIODISK, Solna Sweden). In order to compare the results obtained with BMD and those obtained with Etest, BMD results were rounded up to the next dilution provided by the Etest method. Quality control strains *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were test along with every set of tests.

RESULTS

MIC values were consistently higher for vancomycin (+0.5-1.5 log₂ dilutions) when the isolates were tested by Etest method compared to the broth microdilution method (Table 1).

Table 1. Comparison of broth microdilution method (BMD) and Etest MIC results when testing vancomycin and daptomycin.

Antimicrobial	Log ₂ dilution variation between Etest and reference BMD ^a						
	MIC values (% of 1800 strains):						
	-1	-0.5	0	+0.5	+1	+1.5	+2
Vancomycin	0.0	0.0	0.9	11.1	61.8	26.0	0.2
Daptomycin	0.1	1.8	10.4	33.2	37.0	17.0	0.6

a. BMD MIC results were rounded up to the next dilution value provided on the Etest strip.

When tested by broth microdilution method, the overall (all centers and all years combined) vancomycin MIC mode was 0.75 µg/ml (75.3% of values, rounded up from measured 0.563, 0.625, 0.688 and 0.75 µg/ml), and 96.9% of strains showed vancomycin MIC ≤1 µg/ml. In contrast, when tested by Etest, 58.3 and 32.1% of strains exhibited MIC values of 1.5 and 2 µg/ml, respectively (Figures 1 and 2).

Figure 1. Comparison of reference vancomycin broth microdilution (BMD) MIC results and those provided by and Etest for *S. aureus* (1,800 strains). BMD results were rounded up to the next dilution provided by the Etest method for comparison purposes.

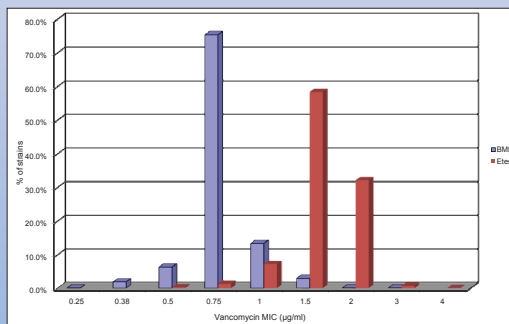
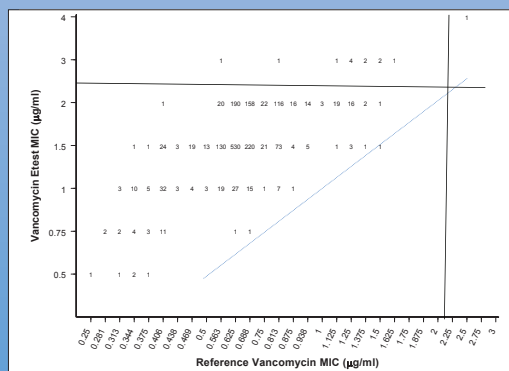


Figure 2. Scattergram showing the correlation between broth microdilution method and Etest MIC results when testing vancomycin. Solid lines indicate CLSI susceptible breakpoint and the broken line indicates complete agreement between the two methods.



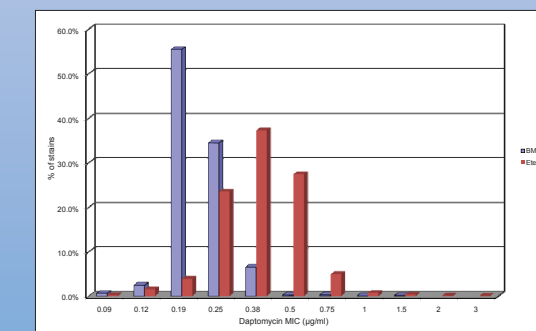
RESULTS

All strains except one showed higher vancomycin MIC when tested by Etest compared to the broth microdilution method. Furthermore, among 13 strains considered non-susceptible (intermediate) to vancomycin by Etest only one had vancomycin MIC > 2 µg/ml by broth microdilution method, a positive predictive value of only 7.7% (Figure 2).

Etest MIC values were also slightly higher for daptomycin (+0.5 - 1 log₂ dilution; Table 1).

When tested by broth microdilution, daptomycin MIC mode was 0.19 µg/ml (55.4% of values, included 0.156 and 0.188 µg/ml), and 92.9% of strains exhibited daptomycin MIC results at ≤0.25 µg/ml (Figures 3 and 4). On the other hand, when tested by Etest, the MIC mode was 0.38 µg/ml (37.2% of values), which is one doubling dilution higher than that obtained with broth microdilution method.

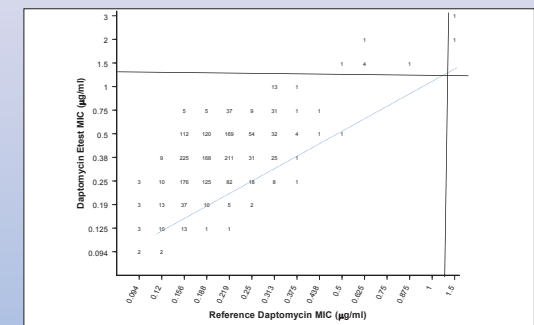
Figure 3. Comparison of reference daptomycin broth microdilution (BMD) MIC results and those provided by and Etest for *S. aureus* (1,800 strains). BMD results were rounded up to the next dilution provided by the Etest method for comparison purposes.



Only 29.2% of strains showed daptomycin MIC at ≤0.25 µg/ml when tested by Etest compared 92.9% when tested by reference broth microdilution method (Figures 2 and 4).

Among nine strains considered daptomycin non-susceptible by Etest only two had non-susceptible MIC result (>1 µg/ml) by reference by broth microdilution method, a positive predictive value of 22.2% (Figure 4).

Figure 4. Scattergram showing the correlation between broth microdilution method and Etest MIC results when testing daptomycin. Solid lines indicate CLSI susceptible breakpoint and the broken line indicates complete agreement between the two methods.



CONCLUSIONS

- Etest provides vancomycin and daptomycin MIC results consistently higher (0.5-1.5 log₂ dilutions) than precisely performed reference broth microdilution methods.
- Susceptibility method used can have a great impact on physician decisions for vancomycin and daptomycin treatment. Dominant population (91.2% of MRSA) would be categorized as vancomycin non-susceptible by Etest method if the susceptible breakpoint was adjusted to ≤1 µg/ml, as suggested by some published clinical outcome studies.
- Daptomycin was less affected (+0.5-1 dilutions and 0.4% false non-susceptible), but could also suffer from perception of falsely elevated MIC values.

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REFERENCES

1. Clinical and Laboratory Standards Institute (2008). M7-A7. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard - seventh edition. Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2008). M100-S18. Performance standards for antimicrobial susceptibility testing: 18th informational supplement. Wayne, PA: CLSI.
3. Fleishman MM, Rousney AS, O'Connell B (2007). Investigation of reduced susceptibility to glycopeptides among methicillin-resistant *Staphylococcus aureus* isolates from patients in Ireland and evaluation of agar screening methods for detection of heterogeneously glycopeptide-intermediate *S. aureus*. *J Clin Microbiol* 45: 3243-3249.
4. Hennes RL, Jorgensen JH (2008). Inhibitory activities of 11 antimicrobial agents and bactericidal activities of vancomycin and daptomycin against invasive methicillin-resistant *Staphylococcus aureus* isolates obtained from 1999 through 2006. *Antimicrob Agents Chemother* 52: 757-760.
5. Hsu DH, Hidayat UK, Cline R, Hendler J, Karlsson A, Yusuf A, Wong-Bertinger A (2008). Comparison of methicillin-specific vancomycin minimum inhibitory concentration values and their predictability for treatment outcome of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. *Int J Antimicrob Agents* 32: 278-285.
6. Makaeles EM, Salako NO, Philip SL, Rotimi VO (2007). Discrepancy in antimicrobial susceptibility test results obtained for oral streptococci with the Etest and agar diffusion. *J Clin Microbiol* 45: 2162-2165.
7. Neoh HM, Hori S, Komatsu M, Oguri T, Takahashi F, Cui L, Hiramoto K (2007). Impact of reduced vancomycin susceptibility on the therapeutic outcome of MRSA bloodstream infections. *Ann Clin Microbiol Infect* 6: 13.
8. Soriano A, Marco F, Martinez JA, Posa E, Amela M, Dinovo VP, Alamo D, Ortega M, Lopez J, Maseda J (2008). Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Dis* 46: 193-200.