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Comparison of Dalbavancin MIC Values Tested Against Gram-Positive Organisms Using Etest (AB BIODISK) and Reference Dilution Methods

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ABSTRACT

Objective: To evaluate the comparative accuracy of the Etest (AB BIODISK, Solna, Sweden) method to determine dalbavancin (DAL) MIC results using the CLSI agar and broth microdilution methods as the reference standards. A total of 100 organisms were tested, all Gram-positive species susceptible (S) to DAL.

Methods: DAL and control vancomycin (VAN) Etest strips were utilized, applying manufacturer technical and interpretive recommendations. CLSI agar dilution (AD) and broth microdilution (BMD) methods were used as the reference method results and all control tests (15 replicates) were within published MIC ranges (M100-S15, 2005). 100 strains were processed as follows: 35 *S. aureus* (SA, 15 MRSA), 20 coagulase-negative staphylococci (CoNS) of 6 species (10 methicillin-resistant), 10 *Enterococcus* spp., 10 *S. pneumoniae* (SPN), 15 beta-haemolytic streptococci (BHS, representing 5 groups), 10 viridans group streptococci and 3 QC strains. DAL was tested by BMD with 0.002% polysorbate 80 by CLSI methods.

Results: All Etest and CLSI reference QC results were within established limits as follows (Etest results only): SA ATCC 29213 (0.032-0.047 mg/L), *E. faecalis* ATCC 29212 (0.094-0.125 mg/L) and SPN ATCC 49619 (0.016-0.023 mg/L). DAL Etest versus reference BMD (table) demonstrated 66% identical results and 98% +/- one $\log_2$ dilution, but versus AD the Etest MICs were generally 2-fold lower with 88 and 98% +/- 2-fold and +/- 4-fold from reference MIC values, respectively. Only BHS showed an Etest modal MIC one $\log_2$ dilution greater than the BMD MIC. The VAN Etest results compared to BMD (72% identical; 100% +/- one $\log_2$ dilution) and to AD (72% identical; 99% +/- one $\log_2$ dilution) MICs also showed excellent correlation.

	DAL Etest MIC variations (Log_2 dilutions) from BMD MIC				
Organism (no.)	-2	-1	0	+1	+2
S. aureus (35)	1	2	28	4	-
CoNS (20)	-	5	12	3	-
Enterococcus spp. (10)	-	2	8	-	-
Beta-haemolytic streptococci (15)	-	1	4	9	1
Viridians group streptococci (10)	-	-	5	5	-
S. pneumoniae (10)	-	1	9	-	-
Total	1	11[a]	66[a]	21[a]	1

a. 98% +/- one $\log_2$ dilution step

Conclusion: DAL Etests provide an excellent and accurate alternative MIC method as demonstrated by these results. Etest could be applied with diagnostic confidence when used with concurrent QC determinations.

INTRODUCTION

The continued emergence of resistance phenotypes among Gram-positive pathogens has compromised the empiric usage of many targeted, traditional antimicrobials and necessitated the development of new agents displaying stability to the most commonly occurring resistance mechanisms. Among agents currently undergoing phase III clinical trials is dalbavancin (formerly BI-397), a once weekly, parenterally-administered semisynthetic glycopeptide whose mechanism of bactericidal activity is similar to that of other glycopeptides. Dalbavancin has been shown to display potent activity against clinically relevant aerobic and anaerobic Gram-positive organisms, including oxacillin (methicillin)-resistant staphylococci, penicillin-resistant *Streptococcus pneumoniae* and certain vancomycin-resistant enterococci (*vanB*/*vanC* phenotypes), and has proven efficacy in clinical trials of skin and soft tissue infections.

The Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution MIC testing methodology and quality control guidelines for dalbavancin have been described previously, and use of dry-form broth microdilution panels for routine laboratory testing has also been validated. Importantly, the incorporation of the surfactant polysorbate-80 into dalbavancin-containing wells in reference frozen-form panels has been found to be critical for accuracy and reproducibility of dalbavancin MIC results.

The purpose of this investigation was to validate the potential use of the Etest (AB BIODISK, Solna, Sweden) as a commercial method for determining susceptibility of indicated bacterial species to dalbavancin, by comparing results with reference MIC values generated by the broth microdilution method, as well as investigating the agar dilution method. Given the familiarity of many laboratories with the Etest and its ease of use, this validation will permit clinical laboratories to test dalbavancin in support of clinical trials and other pre-launch surveillance investigations without having to perform the broth microdilution assay. Disk diffusion has not been established as a test method for dalbavancin, and automated system assays are still in development.

MATERIALS AND METHODS

Organisms Studied: The study organism collection consisted of 100 Gram-positive isolates including *Staphylococcus aureus* (35), coagulase-negative staphylococci (20), *Enterococcus* spp. (10), ß-haemolytic streptococci (15), *Streptococcus pneumoniae* (10), and viridans group streptococci (10). Quality control strains (*S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619 and *E. faecalis* ATCC 29212) were included in each day of testing (five replicates each).

Susceptibility Test Methods: Test methodologies for dalbavancin and vancomycin (class control agent) included broth microdilution, agar dilution (standardized for vancomycin only) and appropriate QC studies, performed according to specifications as described in the CLSI (formerly National Committee for Clinical Laboratory Standards, NCCLS) documents M7-A6, M23-A2 and M100-S16. Broth microdilution testing of dalbavancin included the incorporation of 0.002% polysorbate-80 (final concentration per well) as described in Table 4 of M100-S16. Etest determinations for dalbavancin and vancomycin (control agent) were performed using the manufacturer's recommendations for incubation time, temperature, atmosphere and inoculum concentrations on Mueller-Hinton agar (plus 5% sheep blood for testing of streptococci). MIC values from broth microdilution, agar dilution and Etest methods were generated for all 100 strains tested. All QC replicates for each of the three QC strains were within guidelines as established by the CLSI (data not shown).

RESULTS

- Comparison of vancomycin (control agent) Etest results with broth microdilution and agar dilution results demonstrated a high level of agreement with 72.0% of MIC results being identical, and 100.0% and 99.0% of results being ± one $\log_2$ dilution step, respectively (Tables 1 and 2).
- One coagulase-negative staphylococcus agar dilution result for vancomycin was three $\log_2$ dilutions lower than the Etest result (Table 2).
- Comparison of Etest MIC results with those from broth microdilution for dalbavancin demonstrated very acceptable agreement with 98.0% and 100% of results being ± one and ± two $\log_2$ dilution steps, respectively (Table 1).
- Among organism groups tested against dalbavancin, only the ß-haemolytic and viridans group streptococci demonstrated a slight bias with Etest MIC values being somewhat elevated (approximately one-half of a $\log_2$ dilution step) when compared with broth microdilution (Table 1).
- Dalbavancin agar dilution test results were less comparable to both Etest and broth microdilution MIC results. Only 82% of agar dilution results were within ± one $\log_2$ dilution step, although most (98.0%) were within two $\log_2$ dilution steps (Table 2).
- The bias seen with the agar dilution results for dalbavancin consisted generally of a one $\log_2$ dilution step increase above corresponding Etest values, a trend observed with most tested species or organism groups (Table 2).

Table 1. Variation in dalbavancin and vancomycin MIC ratios comparing Etest (AB BIODISK) MIC results with those of the reference broth microdilution method.

	Etest MIC variations (log_2 dilutions) from BMD MIC:				
Antimicrobial/organism (no. tested)	-2	-1	0	+1	+2
Dalbavancin					
S. aureus (35)	1	2	28	4	-
CoNS (20)	-	5	12	3	-
Enterococcus spp. (10)	-	2	8	-	-
ß-haemolytic streptococci (15)	-	1	4	9	1
Viridians group streptococci (10)	-	-	5	5	-
S. pneumoniae (10)	-	1	9	-	-
Total (100)	1[c]	11[b,c]	66[a,b,c]	21[b,c]	1[c]
Vancomycin					
S. aureus (35)	-	1	32	2	-
CoNS (20)	-	-	12	8	-
Enterococcus spp. (10)	-	-	9	1	-
ß-haemolytic streptococci (15)	-	6	9	-	-
Viridians group streptococci (10)	-	7	3	-	-
S. pneumoniae (10)	-	-	7	3	-
Total (100)	-	14[e]	72[d,e]	14[e]	-

a. 66.0% identical MIC results
b. 98.0% ± one $\log_2$ dilution step
c. 100% ± two $\log_2$ dilution steps
d. 72.0% identical MIC results
e. 100.0% ± one $\log_2$ dilution step

Table 2. Variation in dalbavancin and vancomycin MIC ratios comparing Etest (AB BIODISK) MIC results with those of the agar dilution method.

	Etest MIC variations (log_2 dilutions) from agar dilution MIC:						
Antimicrobial/organism (no. tested)	-3	-2	-1	0	+1	+2	+3
Dalbavancin							
S. aureus (35)	-	5	26	4	-	-	-
CoNS (20)	-	3	12	3	2	-	-
Enterococcus spp. (10)	-	1	4	5	-	-	-
ß-haemolytic streptococci (15)	1	3	10	1	-	-	-
Viridians group streptococci (10)	1	2	5	2	-	-	-
S. pneumoniae (10)	-	2	7	1	-	-	-
Total (100)	2	16[c]	64[b,c]	16[a,b,c]	2[b,c]	-[c]	-
Vancomycin							
S. aureus (35)	-	-	3	32	-	-	-
CoNS (20)	-	-	-	7	12	-	1
Enterococcus spp. (10)	-	-	-	9	1	-	-
ß-haemolytic streptococci (15)	-	-	4	10	1	-	-
Viridians group streptococci (10)	-	-	1	7	2	-	-
S. pneumoniae (10)	-	-	-	7	3	-	-
Total (100)	-	-	8[e]	72[d,e]	19[e]	-	1

a. 16% identical MIC results
b. 82% ± one $\log_2$ dilution step
c. 98% ± two $\log_2$ dilution steps
d. 72% identical MIC results
e. 99% ± one $\log_2$ dilution step

CONCLUSIONS

- The Etest methodology performed extremely well for vancomycin (control agent), with 100% and 99% of results being ± one $\log_2$ dilution step when compared to the broth microdilution and agar dilution antimicrobial test procedures.
- Results from the dalbavancin Etest procedure also demonstrated a high level of agreement (98% ± one $\log_2$ dilution step) with the reference broth microdilution procedure. Results with the as yet unvalidated agar dilution method trended towards higher MIC values.
- Utilization of the dalbavancin Etest method as a surrogate procedure for the broth microdilution assay during investigational studies or after regulatory approval should provide clinical laboratories with a high level of confidence and test accuracy when performing susceptibility procedures on isolates of indicated species.

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