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Multicenter Quality Control Evaluation Results for Dalbavancin (BI397), An Investigational Glycopeptide With Potent Gram-Positive Activity

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ABSTRACT

Background: Dalbavancin (formerly BI397) is a novel glycopeptide agent with a spectrum most resembling teicoplanin, but with documented greater potency than either vancomycin (VANCO) or teicoplanin against all important Gram-positive pathogens. Pharmacokinetic results and Phase II clinical trial success supports once-weekly dosing. This report summarizes the results of an eight laboratory quality control (QC) study for dalbavancin.

Methods: All broth microdilution tests (M7-A6) using four Mueller-Hinton media lots and QC study designs (M23-A2) followed the National Committee for Clinical Laboratory Standards (NCCLS) documents. QC strains tested against dalbavancin included: *Enterococcus faecalis* (EF) ATCC 29212, *Staphylococcus aureus* (SA) ATCC 29213 and *Streptococcus pneumoniae* (SPN) ATCC 49619. VANCO MICs (160 per QC strain) were used as controls.

Results: A total of 320 values were generated per QC organism for each MIC test from the eight laboratories. Proposed three $\log_2$ dilution MIC ranges contained 99.7-100.0% of the reported values (0.03-0.12 μg/ml for EF and SA; and 0.008-0.03 μg/ml for SPN). Internal QC using VANCO MICs showed that 100% of the values (1-4 μg/ml for EF, 0.5-2 μg/ml for SA, and 0.12-0.5 μg/ml for SPN) were within the NCCLS published guidelines. Colony counts performed from the broth microdilution trays showed an average inoculum concentration of 3.5 x 10^5 CFU/ml.

Conclusions: As dalbavancin continues through the clinical trials, these QC guidelines for the broth microdilution test will prove to be valuable for susceptibility test accuracy, since the drug's solubility may limit the utility of disk diffusion methods.

INTRODUCTION

As gram-positive pathogens have acquired resistances to traditional therapeutic agents and multi-drug resistant clones have spread globally, vancomycin in the United States and teicoplanin in Europe have been the antimicrobial agents of choice for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) and invasive enterococcal infections. During recent years, there has been a profound escalation in resistance among these pathogenic bacteria. Vancomycin-resistant enterococci, which cause serious infections including endocarditis, transient and invasive bacteremia and tissue infections, are common and endemic in some institutions in the United States and Europe. As of 2002, a total of eight vancomycin-intermediate *S. aureus* (VISA) from patient infections have been confirmed in the United States. Also in 2002, the first vancomycin-resistant *S. aureus* (VRSA) was isolated in a patient with diabetes, peripheral vascular disease and chronic renal failure. In Europe, teicoplanin has been shown to possess similar spectrum to that of vancomycin, and appears to have fewer side effects which can be advantageous in treating patients with extended treatment regimens. However, the emergence of the confirmed VRSA in the United States and the previously documented teicoplanin intermediate or resistant strains of coagulase-negative staphylococci in Europe, underscores the need for antimicrobial agents which can replace contemporary glycopeptides a "treatment of last resort" for multi-drug resistant gram-positive pathogens.

Dalbavancin (formerly BI397) is a semi-synthetic derivative of a natural glycopeptide, MDL 62,476 (formerly A40926), with a mode of action which disrupts bacterial cell wall biosynthesis. The development of dalbavancin showed great promise because of greater potency compared to either vancomycin or teicoplanin against staphylococci, including some resistant strains. The anti-streptococcal activity of dalbavancin, including penicillin-resistant strains, is similar to that of teicoplanin but superior to vancomycin. The pharmacokinetic properties of dalbavancin are advantageous compared to other glycopeptides due to an extended half-life which allows prolonged dosing intervals, that can be once weekly for some indications.

MATERIALS & METHODS

A quality control (QC) study following the guidelines (M23-A2) and broth microdilution test methods (M7-A6) established by the National Committee for Clinical Laboratory Standards was performed by an eight laboratory study group. The reference frozen-form broth microdilution panels containing four Mueller-Hinton broth lots by quality manufacturers (Difco, Detroit, MI; Hardy, Santa Maria, CA; BBL, Sparks, MD) were supplemented with or without 5% lysed horse blood. Panels were prepared by TREK Diagnostics (Cleveland, OH) and remained frozen at -80ºC until used. The dalbavancin standard powder was obtained from Versicor (Fremont, CA) and vancomycin powder, which was obtained from Sigma Chemical (St. Louis, MO), served as the internal quality control agent. Each laboratory tested three American Type Culture Collection (ATCC) QC strains; *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619 and *S. aureus* ATCC 29213, daily for 10 days (320 MIC values).

The procedure used for testing each of the QC strains is as follows: a 0.5 McFarland standard inoculum was prepared and diluted with one ml into 29 ml of water containing Polysorbate 80. The inoculum suspension was inoculated into the MIC panels using a hand inoculator. The MIC panels were incubated according to NCCLS M7-A6 recommendations. Colony counts were performed from the positive control well after inoculation of the panel to ensure the final inoculum concentration obtained was approximately 5 x 10^5 CFU/ml. The proposed QC ranges were optimized to contain ≥ 95% of all results as recommended by the NCCLS M23-A2 guidelines. Each of the MIC results were tabulated, and compared within and between laboratories. Results from each of the broth media lots were also compared.

Table 1. Inter- and intra-laboratory comparison of dalbavancin MIC results when testing *S. aureus* ATCC 29213 during an eight medical center protocol conforming to NCCLS study guidelines.

	Occurrences by laboratory:								
MIC (μg/ml)	A	B	C	D	E	F	G	H	Total
0.03	5	5							10[a]
0.06	35	33	27	40	38	39	40	38	290[a]
0.12		2	13		2	1		2	20[a]

a. Proposed MIC QC range contains 100.0% of participant results.

Table 2. Distributions of dalbavancin MIC values for all qualifying results from an eight laboratory study using three NCCLS quality control (QC) strains.

	QC organism MIC occurrences:		
	S. aureus	*E. faecalis*	*S. pneumoniae*
MIC (μg/ml)	ATCC 29213	ATCC 29212	ATCC 49619
0.008			22[a]
0.015			250[a]
0.03	10[a]	1[a]	47[a]
0.06	290[a]	277[a]	1
0.12	20[a]	42[a]	

a. Proposed MIC QC ranges contain 99.7 to 100.0% of reported results.

RESULTS

- Table 1 shows an example of the dalbavancin MIC distribution for *S. aureus* ATCC 29213 between the eight laboratories. Over 90% of the combined results were at 0.06 μg/ml, which was also the modal value for all of the eight participating laboratories. The proposed range for *S. aureus* ATCC 29213 is 0.03 - 0.12 μg/ml, which would contain 100% of the reported results.

- Similar results were obtained for *E. faecalis* ATCC 29212 with over 86% of the total results at the modal value (0.06 μg/ml) and a range of 0.03 - 0.12 μg/ml (100% of the reported value).

- For *S. pneumoniae* ATCC 49619, over 78% of the total results were at the modal value (0.015 μg/ml) with a range of 0.008 - 0.03 μg/ml (99.7% of the reported values).

- When the MIC occurrences by media lots were calculated, the same modal values for *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619 (0.06 μg/ml, 0.06 μg/ml, 0.015 μg/ml, respectively) were documented (data not shown).

- Table 2 summarizes all of the proposed QC ranges for *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619 to be used by clinical laboratories for susceptibility testing methods [NCCLS, 2000]. A three $\log_2$ dilution range for the broth microdilution methods would encompass 99.7 - 100.0% of all results produced by the laboratories.

- Concurrent testing using vancomycin as the internal control agent was performed on all QC organisms, with all results (100.0%) within the NCCLS published guidelines (2002); 0.5 - 2 μg/ml for *S. aureus* ATCC 29213, 1 - 4 μg/ml for *E. faecalis* ATCC 29212 and 0.12 - 0.5 μg/ml for *S. pneumoniae* ATCC 49619.

- Colony counts were performed from the broth microdilution panels by subculturing in a quantitative manner onto drug-free plates and the counts ranged from 3 x 10^4 to 1 x 10^6 CFU/ml with an average for all laboratories at 3.5 x 10^5 CFU/ml (target inoculum at 5 x 10^5).

CONCLUSIONS

- This report summarizes the results from a MIC QC study for dalbavancin, a new long acting glycopeptide, tested against the most commonly used gram-positive QC strains.

- The proposed dalbavancin QC guidelines should be utilized during the initial clinical trials, especially since there will be a delay in the development of the disk agar diffusion method.

- These MIC QC ranges when applied, will promote the accuracy of dalbavancin susceptibility testing data for regulatory purposes worldwide.

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