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Background: The CLSI broth microdilution (BMD) method for aerobic Gram-positive strains in calcium-adjusted Mueller-Hinton broth (CAMHB) has been standardized for dalbavancin testing using DMSO and polysorbate-80 (P-80). Similar standardization using the agar dilution method has not been done for dalbavancin. This study was performed to compare dalbavancin BMD and AD results for Gram-positive bacteria. Materials: BMD and AD with 0.002% P-80 was used to test 42 Gram-positive organisms (S. aureus [including ATCC] 29213], coagulase negative staphylococci [CNS], E. faecalis [including ATCC 29212] and streptococci [including S. pneumoniae ATCC 49619]). Vancomycin was tested as a comparator agent. **Results:** In comparison to BMD MICs, dalbavancin AD MICs averaged 2.8 dilutions higher. The number of strains by organism group by dilution difference were:

	Dilution difference (AD-BMD MIC)						
Organism	1	2	3	4	5		
S. aureus		6	7	1			
Staphylococcus (CNS)			3	2	3		
E. faecalis		2		1			
Streptococcus (Beta)	1	7	4	1			

AD MICs for vancomycin were 1 dilution higher than BMD MICs for the majority of the strains. The dalbavancin MIC results (µg/mL) for the three QC strains for BMD and AD methods respectively were: S. aureus ATCC 29213 0.06 and 0.25, E. faecalis ATCC 29212 0.06 and 1, S. pneumoniae ATCC 49619 0.03 and 0.25. Conclusion: Because of the increase in and variability of AD MICs observed in this study, the use the standardized AD or modified AD with addition of P-80 for dalbavancin is not recommended

Introduction

Dalbavancin susceptibility testing has been standardized for broth microdilution (BMD), but not agar dilution (AD). According to the CLSI BMD method (M100-S22), the initial dalbavancin stock solution is made in DMSO to a concentration no higher than 1600 mcg/mL. Intermediate 100x concentrations are diluted in DMSO and the final 1:100 dilutions are made in cation adjusted Mueller Hinton broth (CAMHB)+ 0.002% v/v polysorbate-80 (P-80).

This study was performed to compare dalbavancin BMD MICs to AD MICs to determine if an agar dilution method with addition of P-80 could be recommended. Prior to this study, pilot studies were performed to compare AD MICs using varying amounts of P-80 and these data are also presented.

Methods

Antimicrobial Agents

Dalbavancin Vancomycin

Susceptibility Methods:

CLSI BMD (which includes addition of 0.002% P-80) CLSI AD (with exception of addition of P-80)

Pilot Study Media:

- Cation Adjusted Mueller Hinton Broth (CAMHB) + 0.002% P-80
- Mueller Hinton II Agar (MHA) no P-80 and + P-80 (0.001-2%)

Full Study Media:

- Cation Adjusted Mueller Hinton Broth (CAMHB) + 0.002% P-80
- Cation Adjusted Mueller Hinton Broth (CAMHB) + 5% Lysed Horse Blood
- Mueller Hinton II Agar (MHA) + P-80 (0.002%)
- Mueller Hinton II Agar (MHA) + 5% sheep blood + P-80 (0.002%)

Organisms: Pilot Study: 3 S. aureus: MSSA, MRSA, VISA Full Study: 15 S. aureus, 8 CNS, 5 E. faecalis, 14 Streptococci

Comparison of Broth Microdilution and Agar Dilution Dalbavancin and Vancomycin MIC results for 42 Aerobic Gram-positive Bacteria

Results

Pilot Study (Table 1) :

With addition of 0.001% or 0.002% P-80, dalbavancin MICs were 2-3 higher than broth MICs. With no P-80 and with >0.002% P80, MICs were 4-5 dilutions higher compared to broth MICs. Vancomycin AD and BMD MICs were similar (within 1 dilution).

Full Study (Figures 1 and 2, Table 2): In comparison to BMD MICs, dalbavancin AD MICs were higher:

- 15 S. aureus 2-4 dilutions higher (average 2.6 dilutions higher)
- 8 Coagulase Negative Staphylococci 3-5 dilutions higher (average 4.1 dilutions higher)
- 4 Vancomycin Susceptible *E. faecalis* 2 and 4 dilutions higher (average 2.7 dilutions higher)
- 5 Group A Streptococci 1-3 dilutions higher (average 2.4 dilutions)
- 5 Group B Streptococci 3-4 dilutions higher (average 3.25 dilutions higher)
- 4 Group G and C Streptococci and S. pneumoniae ATCC 49619 3 dilutions higher

Table 1: Pilot Study Results of Dalbavancin MICs (mcg/mL) with Varying P-80 Concentrations in AD Compared to Macrobroth and Compared to Vancomycin BMD and AD MICs for 3 S. aureus.

	Dalbavancin MIC (mcg/mL)									
Study Strains	Macrobroth	Pilot 1 Agar Dilution				Pilot 2 Agar Dilution		Vancomycin MIC (mcg/mL)		
	Macrobrotti	P-80 concentration					P-80 concentration			
	0.002% P80	None	0.002%	0.02%	0.20%	2%	0.001%	0.002%	AD	BMD
001SA (MSSA, ATCC 29213)	0.06	1	0.25	1	1	2	0.25	0.25	1	1
002SA (MRSA)	0.06	1	0.5	1	1	2	0.25	0.12	1	1
003SA (VISA)	1	2	4*	4*	>4**	>4**	0.5*	0.5*	4	8

*Trailing in 1 well prior

**Trailing in 2 wells prior



Figure 2: Mean Dalbavancin BMD and AD MIC Results for Organism Groupings



Conclusions

Dalbavancin AD MICs were consistently higher than broth MICs against Grampositive aerobic organisms

In contrast to BMD, the addition of P-80 to the agar did not reduce dalbavancin MICs and concentrations of P-80 higher than 0.002% actually increased MICs against S. aureus

The use of the standardized (CLSI) AD or modified AD method with the addition of P-80 for dalbavancin is not recommended

MIC>4



Positivo Organisme

Study Strains		Dalbavan	icin MIC	Vancom	ycin MIC	Colony			
		(mcg/mL)		(mcg	g/mL)	Count			
		BMD	AD	BMD	AD	[cfu x 10(5)]			
001SA	(ATCC 29213)	0.06	0.25	1	0.5	3.8			
002SA	MRSA	0.03	0.25	0.5	0.5	3.1			
003SA	VISA	0.25*	1	4	2	2.5			
004SA	MRSA	0.06	0.5	1	0.5	3.3			
005SA	MRSA	0.06	0.5	2	1	5.1			
006SA	MRSA (LZD=R)	0.06*	0.25	1	2	3			
007SA	hVISA	0.12	1	2	2	2.2			
008SA	MRSA	0.12	0.5	2	1	3.5			
009SA	VISA	0.12	0.5	4	4*	4.3			
010SA	VRSA	>4	>4	>64	>64	3.7			
011SA	MSSA	0.12	0.5	2	2	6.2			
012SA	MSSA	0.06	0.5	2	1	5.2			
013SA	MSSA	0.06	0.5	2	2	3.2			
014SA	MSSA	0.06	1	2	4	2.8			
015SA	MSSA	0.03	0.25	0.5	1	3.6			
016CNS	MRSE	0.03	1	2	2	2.8			
017CNS	MRSE	0.03	0.25	1	1	3.1			
018CNS	MRCNS	0.25*	2	1	2	2.2			
019CNS	MRCNS	0.06	2*	2	2	2.6			
020CNS	MSSE	0.03	1	2	4	2.1			
021CNS	MSCNS	0.015	0.25	1	4*	4.8			
022CNS	MSCNS	0.06	1	1	1	2.3			
023CNS	MSCNS	0.03	0.5	1	1	3.1			
024EF	VSE	0.06	0.25	1	1	2.6			
025EF	VSE	0.06	0.25	1	1	5.9			
026EF	VRE	>4	>4	>64	>64	6.1			
027EF	VRE	>4	>4	>64	>64	6.3			
028EF	ATCC 29212	0.06	1	2	4*	3.9			
029GAS	Macrolide R	0.015	0.12	0.5	0.12	5.2			
030GAS	Macrolide R	0.06	0.12	0.5	0.25	4.7			
031GAS	Macrolide S	0.03	0.25*	0.5	0.25	6.1			
032GAS	Macrolide S	0.03	0.12*	0.5	0.25	5.8			
033GAS	SXT R	0.015	0.12	0.5	0.12	5.2			
034GBS		0.06	0.5	0.5	0.5	5.5			
035GBS		0.06	1*	0.5	0.5	4.9			
036GBS		0.06	0.5	0.5	0.5	6.3			
037GBS		0.06	0.5	0.5	0.5	5.1			
038GGS		0.03	0.25	0.25	1	6.5			
039GGS		0.03	0.25	0.25	1	7.1			
040GCS		0.03	0.25	0.5	0.5	6.2			
041GCS		0.03	0.25	0.5	0.5	5.4			
042SP	ATCC 49619	0.03	0.25	0.25	0.5	5.1			

*Trailing in 1 well prior

SA - S. aureus, CNS - Coagulase Negative Staphylococcus, EF - E. faecalis, GAS - Group A Streptococcus, GBS Group B Streptococcus, GCS - Group C Streptococcus, SP - S. pneumoniae

References

- Testing. Approved Standard 22th Edition. CLSI document M100-22 Wayne, PA:

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Table 2: BMD and AD MICs (mcg/mL) for Dalbavancin and Vancomycin for 42 Gram-

. Clinical and Laboratory Standards Institute. 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 9th ed. Approved standard, CLSI M7-09, Wayne, PA. 2. Clinical and Laboratory Standards Institute. 2012. Performance Standards for Antimicrobial Susceptibility