Effect of Serum and Albumin on MIC and MBC of Daptomycin and Comparator Agent for 20 *S. aureus* and 10 Enterococci

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Abstract

Background: The addition of serum and albumin (ALB) to testing media, will affect the free calcium ion concentration (Ca++) which may impact daptomycin in vitro activity. This study was performed to compare daptomycin MIC and MBC data with and without human serum or human ALB with a focus on controlling the final Ca++ concentration.
Methods: The daptomycin MIC and MBC (CLSI broth microdilution) for 30 clinical isolates (20 *S. aureus*, 10 enterococci) and *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212, were obtained using Mueller Hinton Broth supplemented to 50 µg/mL Ca++ (CSMHB), CSMHB + 50% human serum, CSMHB + 4 g/dL ALB, MHB + 50% human serum adjusted to 50 µg/mL Ca++ and MHB + 4 g/dL ALB adjusted to 50 µg/mL Ca++. Vancomycin was also tested using cation adjusted Mueller Hinton broth (CAMHB), CAMHB + 50% human serum and CAMHB + 4 g/dL ALB.
Results:. In MHB/Serum media adjusted to 50 µg/mL Ca++, daptomycin *S. aureus* and enterococci MICs were 4 fold higher than CSMHB MICs. When serum is added, regardless of calcium adjustment, MBCs tend to be 1 dilution higher than MICs. When Ca++ in the CSMHB/ALB was adjusted to 50 µg/mL, *S. aureus* and enterococci MICs were 4 and 4-8 fold higher than CSMHB MICs, respectively. *S. aureus* MBCs were similar to MICs when ALB is added, regardless of calcium adjustment. Vancomycin *S. aureus* MICs were not affected and MBCs increased 1-2 dilutions with addition of serum or ALB.

Media, Ca++ (µg/mL)	Average Daptomycin Results for 20 *S. aureus*		
	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC ratio
CSMHB, 51.4	0.841	0.933	1.15
CSMHB/Serum 62.3	2.639	5.657	2.3
MHB/Serum Calcium Adjusted, 52.2	3.864	8.877	2.4
CSMHB/ALB, 32.8	14.93	18.38	1.3
CSMHB/ALB, Calcium Adjusted, 53.4	3.249	3.864	1.25

Conclusions:
When serum or ALB is added to broth for *in vitro* testing of daptomycin, the Ca++ should be measured and adjusted. Daptomycin MICs, MBCs and MBC/MIC ratios increased with the addition of serum in properly calcium adjusted media. Although *S. aureus* MICs and MBCs also increased with addition of ALB, the MBC/MIC ratios were similar to CSMHB MBC/MIC ratios.

Introduction

Various *in vitro* studies have shown the effect of addition of serum and albumin on daptomycin's bactericidal activity [1-5]. Since free calcium ions (Ca++) will bind to serum proteins and albumin, additional calcium is required in order to simulate physiological levels. This study was performed to provide more extensive MIC and MBC data (compared to prior studies) with and without human serum (50%) or human albumin (at normal serum concentration of 4 g/dL) with a focus on controlling the final Ca++ concentration [Ca++] to physiological levels against 20 *S. aureus* and 10 enterococci.

Materials & Methods

Antimicrobial Agents

Daptomycin:
Cubist Pharmaceuticals, Lot #X2D5549
Concentrations: 0.12 to 128 mg/L

Vancomycin:
MP Biomedicals, Lot #003813j
Concentrations: 0.25 to 256 mg/L

Microorganisms

20 *Staphylococcus aureus:*
Collected from U.S. hospitals between June 2005 and October 2008 and selected to include strains with range of daptomycin MICs from 0.25-2 µg/mL. With the exception of 1 strain, these were methicillin resistant *S. aureus* (MRSA)

10 Enterococci:
Collected from U.S. hospitals between September 2007 and May 2008 and selected to include strains with daptomycin MICs from 1-8 µg/mL. Four strains were vancomycin susceptible *E. faecalis* and 6 strains were *E. faecium*, 4 of which were vancomycin resistant.

Quality Control Strains:
Staphylococcus aureus (ATCC 29213)
Enterococcus faecalis (ATCC 29212)

Media

Abbreviation	Description	[Ca++] µg/mL
CAMHB	Calcium Adjusted Mueller Hinton Broth	20-25
CSMHB	Calcium Supplemented Mueller Hinton Broth	44.9-53.1
+Serum-Ca Adj	CAMHB+50% Serum	52.2
+Alb-Ca Adj	CSMHB+4 g/dL Albumin+20 mg/L Ca++	53.4

Methods

Physiological levels of ionized calcium in serum (approximately 50 mg/L) are obtained if a blood sample is centrifuged in a closed tube and measured immediately. Otherwise [Ca++] in serum may increase as a result of pH changes of the sample due to a substantial loss of CO_2 [8]. When *in vitro* assays are performed with daptomycin in the presence of serum, the serum is typically not freshly collected and is often pooled serum that is processed commercially. In this study, when the pooled serum was added to CSMHB, the [Ca++] was 62.3 mg/L. Therefore, in order better simulate the *in vivo* conditions, the calcium level in the broth had to be lowered so that after addition of the serum the [Ca++] was about 50 mg/L. In this particular study, we were able to achieve physiological levels of calcium with the addition of 50% serum to CAMHB. The addition of 4 g/dL of albumin lowered [Ca++] in the broth and therefore, calcium supplementation was required to achieve 50 mg/L.

MIC and MBC results were determined according CLSI broth microdilution and bactericidal procedures [6-7]. Human serum was heat inactivated prior to use. pH was measured and adjusted to 7.2-7.4 in all broth and broth serum/albumin media prior to making MIC trays.

Protein Binding (PB) by antimicrobial activity [10] = $\frac{\text{+Albumin-Ca Adj MIC} - \text{CSMHB MIC}}{\text{+Albumin-Ca Adj MIC}}$

Results

	Staphylococcus aureus (n=20)		
Daptomycin Test Condition	Geometric Mean MIC Results (µg/mL)	Geometric Mean MBC Results (µg/mL)	Mean MBC/MIC
CSMHB	0.84	0.93 • Similar to MICs except 2 strains 2 fold higher	1.15
+Serum-Ca Adj.	3.86 • 4 fold higher than broth-only MICs	8.88 • 2 fold higher than MICs	2.4
+Albumin-Ca Adj.	3.5 • 4 fold higher than broth-only MICs	3.86 • Similar to the MICs except 5 strains 2 fold higher	1.25
Vancomycin Test Condition	Geometric Mean MIC Results (µg/mL)	Geometric Mean MBC Results (µg/mL)	Mean MBC/MIC
CAMHB	1.07	1.27	1.25
+Serum	1.15	2.64	2.75
+Albumin	1.07	1.74	1.8

	Enterococci (n=10)					
Daptomycin Test Condition	Geometric Mean MIC Results (µg/mL)		Geometric Mean MBC Results (µg/mL)		Mean MBC/MIC	
	Vanco Susc.	Vanco Resist.	Vanco Susc.	Vanco Resist.	Vanco Susc.	Vanco Resist.
CSMHB	3.56	2.0	11.31	6.73	3.17	3.36
+Serum-Ca Adj.	12.70	16.0	28.51	26.91	2.24	1.68
+Albumin-Ca Adj.	17.96	13.45	35.92	26.91	2.0	2.0
Vancomycin Test Condition	Geometric Mean MIC Results (µg/mL)		Geometric Mean MBC Results (µg/mL)		Mean MBC/MIC	
	Vanco Susc.	Vanco Resist.	Vanco Susc.	Vanco Resist.	Vanco Susc.	Vanco Resist.
CAMHB	1.12	362.04*	>256	>256	na	na
+Serum	1.78	430.54**	>256	>256	na	na
+Albumin	1.12	430.54**	>256	>256	na	na

*2 MICs at >256 µg/mL included for mean calculation as 512 µg/mL **3 MICs at >256 µg/mL included for mean calculation as 512 µg/mL

Protein binding by antimicrobial activity was 50-75% (mean = 73.8%) for *S. aureus* and 50-87.5% (mean = 77.6%) for enterococci

Figure 1 Vancomycin *S. aureus* MIC Distibution

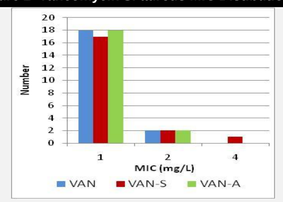
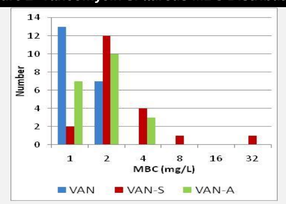
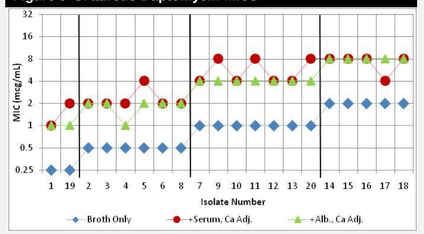


Figure 2 Vancomycin *S. aureus* MBC Distribution

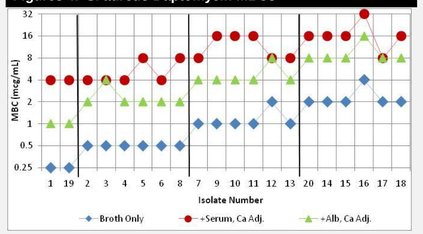


Daptomycin MIC & MBC Results for 20 *S. aureus*

Figure 3 *S. aureus* Daptomycin MICs



Figures 4: *S. aureus* Daptomycin MBCs



Daptomycin MIC & MBC Results for 10 Enterococci

Figure 5 Enterococci Daptomycin MICs

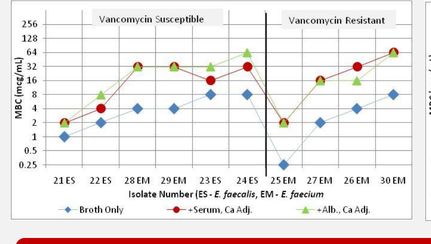
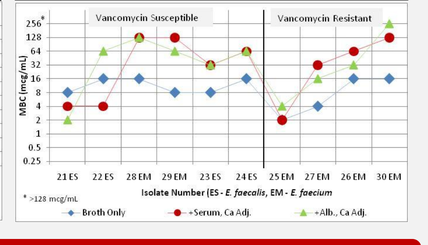


Figure 6: Enterococi Daptomycin MBCs



Conclusions

- The effects of daptomycin and vancomycin on bacterial MICs and MBCs were investigated using human serum and albumin. Because addition of serum and albumin can change free calcium ion levels, they were maintained in this study at approximately physiological levels (50 µg/mL).
- While MICs of vancomycin were not influenced by addition of serum, albumin or calcium ion concentration, the MICs of daptomycin for both *S. aureus* and enterococci were elevated.
- Although the MICs and MBCs increased with addition of albumin, the MBC:MIC ratio for *S. aureus* was similar to the ratio seen with broth alone
- Daptomycin enterococci MBCs, with and without albumin or serum, were considerably lower than vancomycin MBCs.
- The increase in MICs and MBCs seen with addition of serum or albumin are felt to be due to reversible binding (90-93%) to human plasma proteins, primarily to serum albumin [9]. In this study, the protein binding calculated from the *in vitro* activity in presence of albumin was 75%, which corroborates the earlier findings of Craig et al (10). Additional studies of the interaction of daptomycin with serum albumin are warranted.

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