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Comparison of Daptomycin Etest MICs on European Mueller Hinton and IsoSensitest agars against 20 *Staphylococcus aureus*

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Updated Abstract

Background: AB Biodisk recommends BD BBL Mueller-Hinton agar (MHA) for daptomycin (DAP) Etest, since calcium conc. [Ca++] of 25-30 µg/mL are consistently found. There are many brands of MHA and IsoSensitest agar (ISA) used in Europe for the Etest. **Methods:** We studied [Ca++] and DAP Etest MICs using 6 MHA brands: Becton Dickinson (BD), bioMerieux (BM), BioRad (BR), E&O Labs (EO), Mast (MST) and Oxoid (OX) and 3 ISA brands (OX, MST, EO). 20 clinical *S. aureus* isolates, including a large proportion near the susceptible breakpoint (≤1 µg/mL) and *S. aureus* ATCC 29213 were tested by CLSI broth microdilution (BMD) and up to 4 lots of Etest on MHA and 2 lots of Etest on ISA. The agar [Ca++] levels were determined using ion selective electrode method. **Results:** The [Ca++] levels were optimal for BD (30.4) and OX (31.8), lower with MST (19.2) and EO (21.6) and much higher with BM (63.5) and BR (63.6) agar. All non-susceptible (NS) isolates (BMD MICs of 2 µg/mL) had Etest MICs ≥2 µg/mL when tested on MST, BD and OX MHA. Overall, 16.1% and 66.7% of isolates with BMD MICs of 0.25-0.5 µg/mL were NS using OX and MST agar, respectively. Although essential agreement (EA) of BD Etest compared to BMD with susceptible strains with MICs of 1 µg/mL was excellent (100%), 34.1% of Etest MICs were >1 µg/mL (NS). False susceptible results were obtained with BM and BR agar, as MICs were 0.5 to 1 µg/mL dilution lower compared to BMD. Although all NS strains were detected on ISA, 35-59% of susceptible strains with BMD MICs of 1 µg/mL had NS Etest MICs. A shift in Etest performance was noted when MIC results from 2 recent lots were compared to 2 prior lots. **Conclusions:** This study confirms that DAP Etest on BD MHA provides the best correlation to BMD. Because there is no intermediate category, a slight shift in DAP MICs around the breakpoint concentration can result in false NS MICs and therefore, *S. aureus* Etest MICs >1 µg/mL should be confirmed by BMD.

Introduction

- Mueller Hinton Agar (MHA) and IsoSensitest Agar (ISA) are both used in Europe for disk diffusion and Etest antimicrobial susceptibility methods
- There are a variety of manufacturers that provide these agars in Europe
- The cation content in these agars, which includes calcium ion, is not typically measured or controlled by the manufacturers
- The calcium concentration in the agar is an important variable in daptomycin susceptibility testing, including Etest which recommends MHA containing Ca++ = 25-40 µg/mL [1,2]
- This study was performed to compare daptomycin broth microdilution MIC results with Etest MIC results using different brands of prepared MHA and ISA from Europe and Etest lots for a challenge set of 20 *S. aureus.*

Methods

Antibiotics

Etest: (DPC) Daptomycin 0.016 to 256 µg/mL (AB Biodisk, Solna, Sweden) – 4 different lots: BH0645, BH1592, BI0733 and BI0734

Broth Microdilution: Daptomycin 0.03-32 µg/mL (Custom MIC Panel, Trek Diagnostics, E. Grinstead, UK, Cat. No. CMP2DDSL) - Lot #B7195

Media

Etest: Mueller Hinton Agar (MHA), IsoSensitest Agar (ISA) and Isotonic Agar (IST) plates.

Media Type	Manufacturer, Country	Number of lots tested
MHA	Oxoid, UK	4
MHA	E & O, Scotland	1
MHA	bioMerieux, France	2
MHA	BioRad, France	2
MHA	BD, Germany	3
MHA	Mast, UK	1
ISA	Oxoid, UK	4
ISA	E&O, Scotland	1
IST	Mast, UK	1

Broth Microdilution: Trek dried panels with cation-adjusted Mueller Hinton broth (CAMHB, Cat. No. T3462, Lot #148115SA)

Microorganisms

Quality control (QC) strain
Staphylococcus aureus (ATCC 29213)

Stock strains
20 *S. aureus* submitted to Laboratory Specialists, Inc. (the daptomycin reference laboratory that confirms non-susceptible strains from U.S.A. and Canada) were chosen in order to obtain representative strains with BMD MICs near the susceptible breakpoint as follows: 0.25 (3 strains), 0.5 µg/mL (5 strains); 1 µg/mL (8 strains); 2 µg/mL (4 strains)

Calcium analysis of agar and MIC plate broth

- A sample of the prepared agar was weighed and macerated
- A 2:1 volume of sterile water to agar was added and mixed
- The mixture was refrigerated overnight, centrifuged
- The agar supernatant and broth from reconstituted daptomycin wells of the Trek panels were analyzed for Ca++ using ion-selective methodology.

Broth microdilution procedure [3]

- Each strain was tested 4 times (using separate inocula) to verify initial MIC
- Bacterial suspensions in CAMHB (0.5 McFarland standard) were prepared by the direct colony suspension method from blood agar plates incubated for 18–20 hours, and diluted in CAMHB to achieve a final well concentration of 5×10^5 CFU. Colony counts of the final inocula were performed for each replicate.
- 100 µL of each inoculum was dispensed into each well of the MIC panels, and incubated under ambient conditions at 35°C for 24 h.
- The MIC was defined as the lowest drug concentration showing no growth.

Etest procedure [4]

- Each strain was tested with minimally two different lots of Etest strips.
- One of the four inocula used for the BMD replicates was selected for the Etest. Daptomycin Etest strips were applied to the inoculated plates and incubated for 16–18 hours at 35°C.
- Results were read according to the manufacturer's instructions.

Results

Ca++ levels (µg/mL) in MIC in agar (Tables 1-2)

- Reconstituted MIC panel = 50.8
- MHA varied from 19.2 (Mast) to 64.2 (bioMerieux , BioRad)
- ISA/IST = Oxoid ISA: 8.82, 10.47, 8.96; EO ISA: 22.9; Mast IST: 23.5

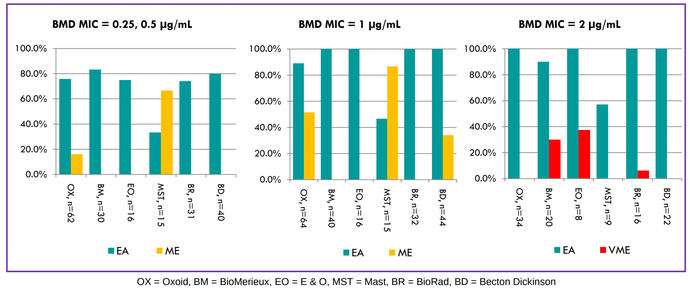
Etest MICs (µg/mL) on MHA (Figure 1, Tables 1-2)

- Non-susceptible (NS) strains (BMD MICs = 2):
  100% Category Agreement (CA) - all Etest MICs were ≥1.5 (NS) with BD, OX, MST
  Very Major Errors (VME)–30.0, 37.5 and 6.3% of Etest MICs were ≤1 (susceptible) with BM, EO and BR respectively
- Susceptible (S) strains (BMD MICs = 1):
  100% CA - all Etest MICs were in susceptible range with BM, EO, BR
  Major Errors (ME) –51.6, 86.7 and 34.1% of Etest MICs were ≥1.5 (NS) with OX, MST and BD, respectively.
- Susceptible strains (BMD MICs = 0.25, 0.5):
  100% CA - all Etest MICs were in susceptible range with BM, EO, BR, BD
  ME –16.1 and 66.7% of Etest MICs were ≥1.5 (NS) with OX and MST, respectively
  Etest Lot Variation – Etest MICs were 0.5 to 2 dilutions lower with Etest lots BI0733, BI0734 compared to lots BH0645, BH1592
- *S. aureus* ATCC 29213 (Table 2): Etest MICs of 0.125 (one dilution below the CLSI QC range) were obtained using MHA with appropriate levels of Ca++ (OX and BD)

Etest MICs on ISA and IST

- Non-susceptible strains (BMD MICs = 2):
  100% CA - there were no VME
- Susceptible strains (BMD MICs = 1):
  ME – 85.7, 85.7 and 92.9% of Etest MICs were ≥1.5 (non-susceptible) with OX, EO and MST, respectively.
- Susceptible strains (BMD MICs = 0.25, 0.5):
  100% CA - all Etest MICs were in susceptible range with EO
  ME –18.8 and 12.5% of Etest MICs were ≥1.5 (non-susceptible) with OX and MST, respectively

Figure 1: Comparison of Essential Agreement (EA) and Major Error (ME) and Very Major Error (VME) Rates by MHA Manufacturer



OX = Oxoid, BM = BioMerieux, EO = E & O, MST = Mast, BR = BioRad, BD = Becton Dickinson

Table 1: Dilution Difference of Etest MIC Compared to BMD MIC by European Supplied Mueller Hinton Agar and Etest Lot

		BMD MIC = 0.25, 0.5 µg/mL									BMD MIC = 1 µg/mL									BMD MIC = 2 µg/mL						
MHA Mfr (lot no.) [Ca++] µg/mL	Etest	≥-2	-1.5	-1	-0.5	0	0.5	1	1.5	≥2	≥-2	-1.5	-1	-0.5	0	0.5	1	1.5	≥2	-1.5	-1	-0.5	0	0.5	1	1.5
OX (1041603) Ca++ = 31.0	BH0645					1		3						1		1	4					1	1	1		
	BH1592				1		2	1							2	3	1						3			
	BI0733	1	1	1	1										1	1							1			
	BI0734			3												2							1			
OX (1041233) Ca++ = 30.4	BH0645					1		2	1					1		3	2					2	2			
	BH1592				1		2	1						1		3	2					2	2			
	BI0733	1	1	1	1										1	1						1				
	BI0734	1		3												2						1				
OX (1043019) Ca++ = 36.7	BH0645						1	1	2							1	5						1	2		
	BH1592						1	1	2							2	3	1					1	2		
	BI0733			1	1	1		1								1	1						1			
	BI0734			1		1	1	1									2						1			
OX (1042711) Ca++ = 29.1	BH0645					1			3						1		4	1						3		
	BH1592						1	1	2							3	2	1						3		
	BI0733			1	1			1										2					1			
	BI0734			1		2	1											2					1			
BM 817871501 Ca++ = 62.7	BH0645				2	1							2	3	1					1		1	1			
	BH1592			1	2	1							2	3	1					1		2				
	BI0733	1	2	1									1		1							1				
	BI0734	2		2									1		1							1				
BM 817870101 Ca++ = 63.6	BH0645			1	2	1							3	2	1						1	2				
	BH1592			2	1	1							4	1	1						1	2				
BM 817869301 Ca++ = 64.2	BH0645				2	1							4	1	1						1	2				
	BH1592				3	1							5		1						1	2				
EO 08068857 Ca++ = 21.6	BH0645					3	1							2	2	2					1		2			
	BH1592					3	1							1	5							1	2			
	BI0733	2		2											2						1					
	BI0734	2		2											2						1					
MST 227702 Ca++ = 19.2	BH0645							1	3								3	2	1				1			2
	BH1592								4							2	1	2	1						1	1
	BI0733				1			1	2										1					1		
	BI0734			1			1	1									1	1						1		
BR 8A2466 Ca++ = 63.0	BH0645					2		1							4	2						1	2			
	BH1592				1	1	2							1	3	2						1	2			
	BI0733	1	1	1		1								1		1						1				
	BI0734	2		2											1	1						1				
BR 8A2465 Ca++ = 64.2	BH0645					2	2								4	2						1	2			
	BH1592					3	1							1	4	1					1		2			
	BI0733	1	1	2											1	1						1				
	BI0734	2		2											1	1						1				
BD 8032989 Ca++ = 29.4	BH0645					1	3							2	2		2					1	1	1		
	BH1592					1	1	2						1	2	1	2					1		2		
BD 8050818 Ca++ = 30.6	BH0645						2	2							1	3	2						1	2		
	BH1592						1	3								4	2					1		2		
	BI0733	1	1			2									1	1							1			
	BI0734	2			1	1									1	1							1			
BD 8044806 Ca++ = 31.4	BH0645					1	1	2							2	2	2					1	2			
	BH1592					1	1	2							3	2	1						2	1		
	BI0733	2			1	1									1		1						1			
	BI0734	2		1		1									1		1						1			

Within Essential Agreement | Major Error | Very Major Error

OX = Oxoid, BM = BioMerieux, EO = E & O, MST = Mast, BR = BioRad, BD = Becton Dickinson

Table 2: *S. aureus* ATCC 29213 Daptomycin MIC by Etest and MHA Lots

MHA Mfr., Lot # ([Ca++] µg/mL)	Etest Lot Number					
	Lot #BH0645		Lot #BH1592		Lot #BI0733	Lot #BI0734
OX, 1041603 (31.0)	0.25	**0.125**	0.25	**0.19**	**0.125**	**0.125**
OX, 1041233 (30.4)	0.25	0.25	0.25	**0.19**	**0.125**	**0.125**
OX, 1043019 (36.7)	0.38	0.25	0.25	0.25	**0.19**	**0.19**
OX, 1042711 (29.1)	0.25	0.25	0.38	0.38	0.25	0.25
BM, 817871501 (62.7)	**0.125**	**0.125**	**0.125**	**0.125**	**0.094**	**0.094**
BM, 817870101 (63.6)	**0.125**	**0.125**	**0.125**	**0.094**	N/A	N/A
BM, 817869301 (64.2)	**0.094**	**0.094**	**0.094**	**0.125**	N/A	N/A
EO, 08068857 (21.6)	**0.19**	**0.125**	**0.19**	**0.125**	**0.094**	**0.125**
MST, 227702 (19.2)	0.5	0.75	0.38	0.75	0.38	0.5
BR, 8A2466 (63.0)	**0.125**	**0.125**	**0.125**	**0.094**	**0.094**	**0.094**
BR, 8A2465 (64.2)	**0.125**	**0.125**	**0.094**	**0.094**	**0.094**	**0.064**
BD, 8032989 (29.4)	**0.125**	**0.125**	0.25	**0.125**	N/A	N/A
BD, 8050818 (30.6)	0.25	0.25	0.25	0.25	**0.125**	**0.19**
BD, 8044806 (31.4)	0.25	**0.125**	**0.19**	0.25	**0.125**	**0.125**

OX = Oxoid, BM = BioMerieux, EO = E & O, MST = Mast, BR = BioRad, BD = Becton Dickinson

Bold Print: Outside CLSI *S. aureus* QC range 0.25 - 1 µg/mL

Conclusions:

- There was considerable variation of [Ca++] in the European commercially prepared media . Of the MHA tested, BD and Oxoid MHA were the only brands with 25-40 µg/mL , as currently recommended by Etest manufacturer.
- Etest MICs on bioMeriuex, E&O, and BioRad MHA failed to detect some non-susceptible strains.
- Etest MICs on Oxoid and Mast MHA and all IsoSensitest/Isotonic agars provided false resistant results among strains with typical MICs of 0.25-0.5 µg/mL.
- The daptomycin QC range for *S. aureus* 29213 requires further evaluation . MICs using BD MHA and current Etest lots were below CLSI range.
- Etest using BD MHA provided the best correlation to BMD, however 34.1% of test results with isolates having BMD MICs = 1 µg/mL were considered NS by Etest. Due to this observed discrepancy, the lack of an intermediate category and the rare occurrence of *S. aureus* isolates with MICs of 1 (approximately 1% of surveillance isolates), we recommend validation of NS Etest results (MIC = 1.5 or 2 µg/mL) by repeating with gold standard BMD.

Acknowledgement
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References
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3. CLSI document M7–A7. Vol 26, No. 2. 4. Etest PI (add number)