Comparison of Daptomycin Etest MICs with Broth Microdilution MICs against a Challenge Set of 58 *S. aureus* and 44 *Enterococcus spp*

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Abstract

Background: The purpose of this study was to evaluate the performance of daptomycin Etest, recently FDA cleared, against challenge strains (laboratory derived and clinical isolates) with MICs bordering the susceptible breakpoint.

Methods: 44 *Enterococcus faecalis* and *E. faecium* (including 29 non-susceptible strains) and 58 *Staphylococcus aureus* (including 43 non-susceptible strains) were tested by broth microdilution (BMD) (Trek) and by Etest using Mueller Hinton Agar (MHA) from three different manufacturers on two separate days. Quality control strains S. aureus (ATCC 29213) and *E. faecalis* (ATCC 29212) were tested on each day of testing.

Results: There was good correlation of *S. aureus* MICs with all media. Although nearly all enterococci MICs were within 2 dilutions of one another, category agreement rates were less than 90% as a result of Etest MICs that tended to be ½ to 1 dilution lower than BMD MICs. The agreement rates of Etest MICs to BMD MICs were:

Organism		% Agreement		
MHA	n	+/- 1 dilution	+/- 2 dilutions	Category
S. aureus				
BD	116	97.5	100	94.8
Remel	116	94.8	100	93.1
Hardy	82	97.6	100	97.6
Enterococci				
BD	88	86.9	100	85.2*
Remel	88	79	97.4	78.4*
Hardy	58	80.8	100	62.1

**E. faecalis* (n=22) = 95.4% (BD) and 100% (Remel)

Conclusions: There was good correlation of Etest results with BMD against the challenge *S. aureus* and *E. faecalis* strains. The slight difference in Etest MICs, primarily with *E. faecium*, impacts interpretive results for strains with MICs near the breakpoint and highlights the need for a daptomycin intermediate interpretive category.

Introduction

The daptomycin Etest was recently FDA cleared and made available for clinical laboratory testing of staphylococci and enterococci. This study was conducted in order to compare reference microbroth dilution and Etest MICs of challenge strains. The challenge strains consisted of both laboratory derived and clinical isolates with the majority of MICs bordering the susceptible breakpoint.

Methods

Mueller Hinton Agar (MHA)	Lot Number	Calcium conc. * (mg/L)
Becton Dickinson (BD), Sparks, MD	5005933	24.1
Remel, Lenexa, KS	564028	35.0
Hardy, Santa Maria, CA	05060	30.1

* Samples of Mueller Hinton agar were tested for free calcium content utilizing ion electrode methodology. (Laboratory Specialists, Inc., Westlake, OH)

Daptomycin Etest (AB Biodisk, Solna Sweden):

Cat. No.: DPC, Lot No. BF0617.

The daptomycin Etest strip has been developed to contain both the exponential daptomycin gradient and a constant Ca^{2+} level, allowing the daptomycin Etest strip to be used on commercially-available MHA without regards to the media's inherent Ca^{2+} content.

Isolates:

102 Laboratory and clinically derived strains with the following MICs (number of strains at each MIC):

Species	MIC (mcg/mL) 0.25	0.5	1	2	4	8	16	32	>32
S. aureus (n=58)	2	8	5	17	2	3	1	1	
E. faecium (n= 33)	1		1	3	7	11	1	6	3
E. faecalis (n=11)			2		2	1	3		3

QC Strains: *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 29213) were tested on each day of testing.

Microbroth Dilution MICs: According to NCCLS/ CLSI

Etest MICs: according to Etest manufacturer's procedures.

Results

***S. aureus* Etest MICs (Figures 1 and 3)**

For all media, Essential agreement rates were 95% (within +/- 1 dilution compared to reference MICs). Categorical agreement rates were >93%.

The same MIC was achieved most often with BD media. The percentage of strains with the same MIC* compared to microbroth dilution were:

Replicate	BD	Remel	Hardy
1	62	60.4	46.4
2	56.9	56.9	48.8

*Based on rounding Etest MICs to next highest doubling dilution

Etest MICs tended to be 0.5 dilutions (on average) lower than microbroth MICs. The percentage of strains with MICs 1 dilution or more lower than microbroth dilution were:

Replicate	BD	Remel	Hardy
1	18.9	36.1	51.2
2	25.8	39.6	48.8

Category Discrepancies (Table 1)

Two strains of the 58 isolates tested were non-susceptible by microbroth and susceptible by Etest and one strain was susceptible by microbroth and non-susceptible by Etest using BD agar. In all cases, the difference in MICs was 1 dilution.

Enterococci Etest MICs (Figures 2 and 4)

For all media, Essential agreement rates were > 79% (within +/- 1 dilution compared to reference MICs). Categorical agreement rates were >62%.

Agreement rates were higher for *E. faecalis* (category agreement rate >94.5%).

The same MIC was achieved most often with BD media. The percentage of strains with the same MIC* compared to microbroth dilution were:

Replicate	BD	Remel	Hardy
1	52.7	36.9	26.9
2	39.5	23.7	26.9

*Based on rounding Etest MICs to next highest doubling dilution

Etest MICs tended to be 1 dilution (on average) lower than microbroth MICs. The percentage of strains with MICs 1dilution or more lower than microbroth dilution were:

Replicate	BD	Remel	Hardy
1	68.4	76.3	96.2
2	76.2	84.3	100

Category Discrepancies (Table 1)

Using BD agar, 8 strains were non-susceptible by microbroth and susceptible by Etest and one strain was susceptible by microbroth and non-susceptible by Etest. The difference in MICs was 0.5 to 1 dilution with most strains.

Conclusions

- Etest performed well compared to microbroth dilution against a challenge set of *S. aureus*
- Etest enterococci MICs were on average one dilution lower than microbroth dilution
- Best correlation (staphylococci and enterococci) was obtained with BD MHA
- An intermediate susceptibility category would provide a needed buffer zone for daptomycin testing to accommodate slight method variations.

Figure 1: Frequency distribution of *S. aureus* MICs by Etest on 3 different MHA (BD, Remel and Hardy) and reference microbroth dilution MIC. Etest MICs rounded to next doubling dilution.)

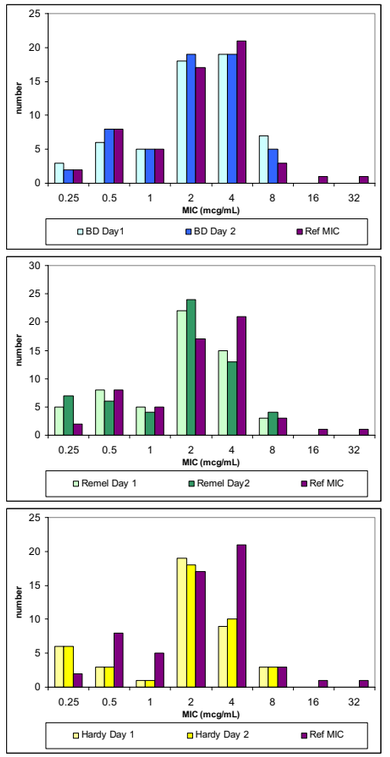

Figure 2: Frequency distribution of enterococci MICs by Etest on 3 different MHA (BD, Remel and Hardy) and reference microbroth dilution MIC (Etest MICs rounded to next doubling dilution.)

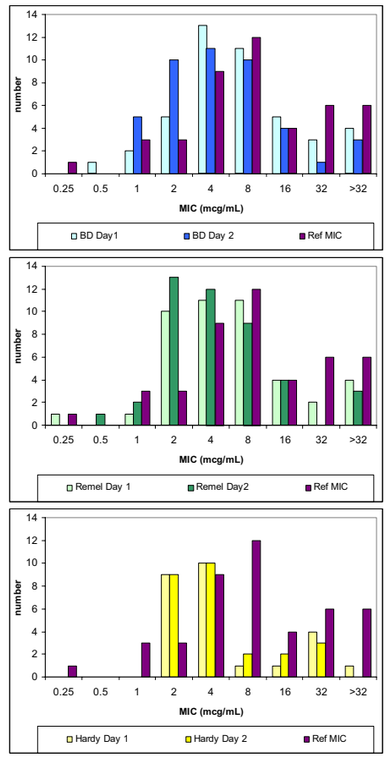

Figure 3: Comparison of *S. aureus* daptomycin Etest MICs on three different MHA to microbroth dilution MICs: percentage dilution difference

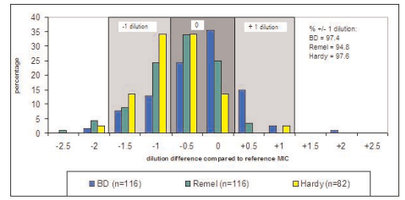

Figure 4: Comparison of enterococci daptomycin Etest MICs on three different MHA to microbroth dilution MICs: percentage dilution difference

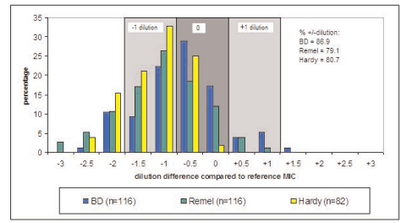

Table 1: Category discrepancies
(discrepant MICs in red print)

Strain No.	Reference MIC (mcg/mL)	Etest (mcg/mL) BD	Remel	Hardy
Reference MIC = Susceptible, Etest = Non-Susceptible				
S. aureus:				
SA093	1	2,2	1,1	NA
Reference MIC = Non-Susceptible, Etest = Susceptible				
S. aureus:				
SA029	2	2,2	1,0.75	1,1
SA090	2	1.5,1	1.5, 1.5	NA
SA092	2	2,2	1,1.5	NA
SA094	2	1.5, 2	1,1	NA
SA095	2	1,1	1,0.75	NA
E. faecalis:				
ES057	8	6,6	8,6	3,4
ES079	16	4,6	6,6	NA
E. faecium:				
EM003	8	8,8	4,4	4,4
EM031	8	3,2	2,2	2,2
EM032	8	3,4	3,3	3,3
EM035	8	4,4	3,4	3,4
EM052	16	8,4	8,2	4,4
EM055	8	6,4	4,3	3,3
EM058	8	6,4	4,2	4,3
EM064	8	6,6	6,4	4,3
EM066	8	4,3	6,2	3,2

NA – Not Available