Comparison of Daptomycin Etest MICs on European Mueller Hinton and IsoSensitest agars against 20 Staphylococcus aureus
L. M. KOETH1, J. DIFRANCO 1
1Laboratory Specialists, Inc., Westlake, OH

Introduction
- Mueller Hinton Agar (MHA) and IsoSensitest Agar (ISA) are both used in Europe for disk diffusion and Etest antimicrobial susceptibility methods
- There is a variability of manufacturers that produce these agars in Europe
- The calcium content in these agars, which includes calcium oxalate, 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)

Methods

Antibiotics

Etest (BD) Daptomycin 0.15 to 256 µg/mL (AB Biodisk, Solna, Sweden) - 4 different lots: BM 817891, BM 817892, BM 817893
Reconstituted MHA: Daptomycin 0.03-32 µg/mL, Custom MIC Panel, Trek Diagnostics, E. Grinstead, UK, Cat. No. CMP2DDSL)

Etest MICs on MHA (Tables 1-2)
- Non-susceptible (NS) strains (MHC MIC > 2 µg/mL)
- Susceptible strains (MHC MIC ≤ 2 µg/mL) using BD MHA and current Etest lots were below CLSI range.
- Etest MICs on ISA and IST
- Non-susceptible strains (MHC MIC > 4 µg/mL)
- Susceptible strains (MHC MIC ≤ 4 µg/mL)

Conclusions
- There was considerable variation of Ca++ in the European commercially prepared media for daptomycin
- Etest MICs on BD and Oxoid MHA were the only brands with 25-40 µg/mL, as currently recommended by Etest manufacturer.
- Etest MICs on Oxoid and Mast MHA failed to detect some non-susceptible strains.
- Etest MICs on Oxoid and Mast MHA and all IsoSensitest/Ist agar plates provided false resistant results among strains with typical MHC MICs of 0.25-6.0 µg/mL.
- The daptomycin QC range for 8. aureus29231 requires further evaluation: Etest MICs using BD MHA and current Etest lots were below CLSI range.
- Etest using BD MHA provided the best correlation to BMD, however 34% of test results with isolates having BMD MICs > 1 µg/mL were considered NS by Etest. This may be due to false resistant results among strains with typical MHC MICs of 0.25-6.0 µg/mL.

Calcium analysis of agar and MIC plate broth
- Assay of the prepared agar plates and reconstituted broth
- A 2:1 volume of sterile water to agar was added and mixed
- The broth was refrigerated overnight, centrifuged
- The agar supernatent and broth from reconstituted daptomycin wells of the Trek panels were analyzed for Ca++ using ion-selective methodology.

Bacterial suspensions in CAMHB (0.5 McFarland standard) were prepared by the direct colony suspension method from blood agar plates incubated for 18-22 hours, and diluted in CAMHB to achieve a final well concentration of 5x10⁶ CFU/mL. Colony counts of the final inocula were performed near the susceptible breakpoint (≤ 1 µg/mL) and 5x10⁶ CFU/mL. All colonies were rehydrated into each well of the MIC panels, and incubated under ambient conditions at 25°C for 24 h. The MIC was defined as the lowest drug concentration showing no growth.

Etest procedure (3)
- Each strain was tested with minimally two different lots of Etest strips.
- The calcium level of the agars used were 30 µg/mL. The Etest results with isolates having BMD MICs = 2 were read according to the manufacturer’s instructions.

Daptomycin Etest MICs on European Mueller Hinton and IsoSensitest agars

Results

Etest procedure (4)
- Conclusions: The study confirms that DAP Etest on BD MHA provides the best correlation to BMD. Although all NS strains were confirmed by BMD, there were no VME.

References
1. KOETH, L. USA. 2004; 21:27-34
2. Etest M0003091 100161 C01 014 DPC