P1890
14th ECCMID
Prague
1 — 4 May 2004

CHIRON

QC study evaluating the performance of daptomycin and combination daptomycin/calcium disks and Etest

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ABSTRACT

Background: *In vitro* susceptibility testing of daptomycin, a cyclic lipopeptide antibiotic with good antimicrobial activity against most Gram-positive bacteria, requires the presence of physiological levels of calcium ions in the broth media. Previous studies have shown that agar calcium levels as low as 25 mg/L have been adequate for disk diffusion testing. This study was performed to evaluate five commercial agars and to determine the efficacy of both disks and Etest strips containing a combination of daptomycin and calcium.

Methods: We tested each of the QC strains, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212, on multiple days using Mueller Hinton agar (MHA) from BD, Remel and Hardy, Mast Isotonic agar supplemented with 50 mg/L of calcium (ISTA), and Oxoid Isosensitest agar (ISOA). Thirty microgram daptomycin disks/tablets from each of the four different manufacturers, BBL (BD), Oxoid (Remel), Mast (Hardy) and Rosco were tested. In addition, Mast disks with 20, 40, 60, 80 and 100 mcg calcium and Etest strips with daptomycin, daptomycin + 40 mg/L calcium and daptomycin + 50 mg/L calcium were evaluated. Calcium testing of each medium was performed using an ion selective electrode.

Results: Mean zone diameters (mm) for *S. aureus* 25923 and mean Etest MICs (mg/L) for *S. aureus* 29213 were:

	Study 2		Study 1			
	BD MHA	Remel MHA	BD MHA	Hardy MHA	Oxoid ISOS	Mast ISOT
Disk – *S. aureus* 25923 (expected = 18-23 mm)						
Oxoid	18.6	20.75	-	-	-	-
BBL	18.75	21.05	20.19	21.09	15.28	22.17
Rosco	17.75	19.5	19.84	21.5	12.91	25
Mast	17.5	19.4	19.25	20.06	12.38	21.33
Mast+100	19.5	20.75	21.19	21.03	19.56	25.53
Rosco+100	20	20.8	22.06	22.44	20.94	27.42
Etest – *S. aureus* 29213 (expected = 0.25-1 mcg/mL)						
Dap	1.68	0.84	1.46	0.88	8.95	0.18
Dap+40	0.74	0.47	-	-	-	-
Dap+50	0.35	0.23	0.35	0.19	0.4	0.08

Conclusions: Disks containing daptomycin alone from BD, Rosco, and Mast resulted in QC values in range for all media except ISOS. With the exception of ISOT, the addition of 100 mcg of calcium to daptomycin Mast disks and Rosco tablets provides for QC results within range for all media including ISOA. The two different lots of BD from studies 1 and 2 affected the MICs and zone diameters. Etest containing daptomycin and 40 mcg of calcium provided optimal QC results.

INTRODUCTION

Daptomycin is the first antibiotic in a new structural class – the cyclic lipopeptides. Daptomycin exerts potent bactericidal activity against Gram-positive bacteria, including multi-resistant strains by virtue of a novel mode of action in which the bacterial cytoplasmic membrane is depolarized resulting in release of intracellular potassium ions.

The *in vitro* activity of daptomycin is influenced by the local concentration of calcium ions (Ca^{2+}). Broth testing standards have been developed with the incorporation of physiological levels of Ca^{2+} (i.e. 50 mg/L). However, no calcium concentration or testing standards have been developed with the agar dilution method.

Consistency of susceptibility results using different media and antibiotic containing disks is important for informed antimicrobial prescribing and for comparisons between laboratories and surveillance studies of resistance trends.

This study was undertaken to evaluate the effects of five commercial agars upon determination of susceptibility tests using Etest strips and a range of antibiotic disks containing 30 mcg daptomycin, with some products also containing increasing concentrations of calcium.

MATERIALS AND METHODS

Media

The following five commercial media were tested:

Media	Abbreviation	Study No/Lot No	Manufacturer
Mueller Hinton Agar	MHA	1/3107324, 3107307	BD
Mueller Hinton Agar	MHA	2/3282384	BD
Mueller Hinton Agar	MHA	1/3106, 3132	Hardy
Mueller Hinton Agar	MHA	2/395733	Remel
Isotonic Agar*	ISOT	1/142770, 142070	Mast
Isosensitest Agar	ISOS	1/286947	Oxoid

* Supplemented with 50 mg/L of calcium

The free calcium (Ca^{2+}) concentration of each medium was determined using an ion selective electrode.[1]

Disks

Thirty microgram daptomycin disks/tablets from each of the four different manufacturers, BBL (BD), Oxoid (Remel), Mast (Hardy) and Rosco were tested. In addition, Mast disks with 20, 40, 60, 80 and 100 mcg calcium and Etest strips (AB Biodisk) with daptomycin, daptomycin + 40 mg/L calcium and daptomycin + 50 mg/L calcium were evaluated.

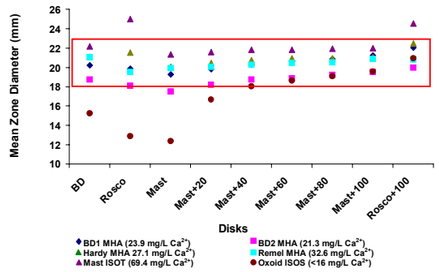
QC strains

Three QC strains, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212, were used. Each strain was tested on multiple days.

Disk testing and Etest methods

Zones of inhibition around disks and Etest strips were determined according to NCCLS guidelines[2] and manufacturers' recommendations, respectively, for each combination of media, disk (or Etest strip) and QC strain on multiple days.

Figure 1. Mean zone diameters for *S. aureus* ATCC 25923

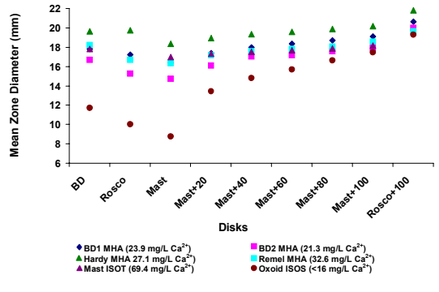


Footnotes
1. Red Box indicates NCCLS expected QC range of 18-23 mm
2. Data not shown. Mean zone diameters for Oxoid disks on Mueller Hinton Agar were: BD2 = 18.60 mm Remel = 20.75 mm
3. BD1 MHA n=32, BD2 MHA n=20, Hardy MHA n=32, Remel MHA n=20, Mast ISOT n=17, Oxoid ISOS n=32

Figure 2. Mean zone diameters for *S. aureus* ATCC 29213

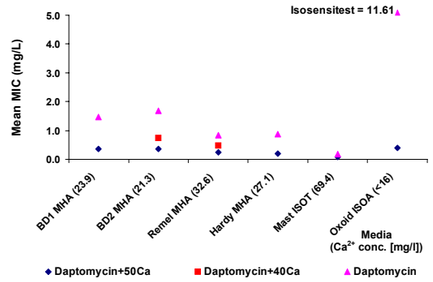
Footnotes
1. Data not shown. Mean zone diameters for Oxoid disks on Mueller Hinton Agar were: BD2 = 19.40 mm Remel = 20.55 mm
2. BD1 MHA n=32, BD2 MHA n=20, Hardy MHA n=32, Remel MHA n=20, Mast ISOT n=17, Oxoid ISOS n=32

Figure 3. Mean zone diameters for *E. faecalis* ATCC 29212



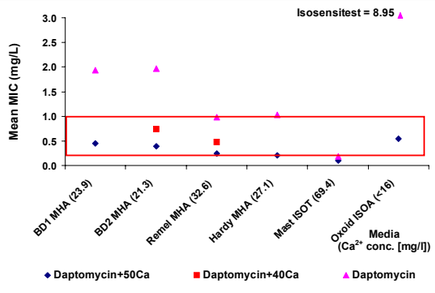
Footnotes
1. Data not shown. Mean zone diameters for Oxoid disks on Mueller Hinton Agar were: BD = 15.55 mm Remel = 17.50 mm
2. BD1 MHA n=26, BD2 MHA n=20, Hardy MHA n=26, Remel MHA n=20, Mast ISOT n=11, Oxoid ISOS n=26

Figure 4. Mean MICs for *S. aureus* ATCC 25923



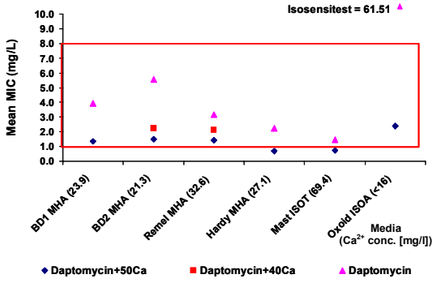
Footnote.
BDI MHA n=32, BD2 MHA n=20, Remel n=20, Hardy MHA n=32, Mast ISOT n=17, Oxoid ISOS n=32

Figure 5. Mean MICs for *S. aureus* ATCC 29213



Footnotes
1. Red Box indicates NCCLS expected QC MIC range of 0.25 – 1 mg/L
2. BD1 MHA n=32, BD2 MHA n=20, Remel MHA n=20, Hardy MHA n=32, Mast ISOT n=17, Oxoid ISOS n=32

Figure 6. Mean MICs for *E. faecalis* ATCC 29212



Footnotes
1. Red Box indicates NCCLS expected QC MIC range of 1-8 mg/L
2. BD1 MHA n=26, BD2 MHA n=20, Remel MHA n=20, Hardy MHA n=26, Mast ISOT n=11, Oxoid ISOS n=26

RESULTS

Calcium concentrations in media

- Calcium concentrations in the test media ranged from 11.09 and 15.17 mg/L in two lots of Oxoid Isosensitest Agar to 69.4 mg/L in Mast Isotonic Agar. The calcium concentration in Mueller Hinton agars ranged from 21.3 — 32.9 mg/L (i.e. 23.94 mg/L and 21.3 mg/L being recorded for BD Studies 1 and 2, 1 mg/L for Hardy and 32.6 mg/L for Remel agars).

Disk tests

***S. aureus* ATCC 25923 (Figure 1)**

- The NCCLS expected range of zone diameters is 18 – 23 mm.
- All disks gave satisfactory zone diameters on Mueller Hinton Agars from BD (Study 1 only), Hardy and Remel. The mean zone diameter for BD (Study 2) with MAST disks was slightly below the expected range (17.50 mm).
- Oxoid Isosensitest Agar (low calcium concentration, <16 mg/L Ca^{2+}) resulted in unacceptably small zone diameters with BD, Rosco, Mast and Mast 20 disks.
- Mast Isotonic Agar (69.4 mg/L Ca^{2+}) resulted in unacceptably large zone diameters with Rosco and Rosco 100 disks.

***S. aureus* ATCC 29213 (Figure 2)**

- The pattern of zone diameters with respect to media and disks, exhibited by *S. aureus* ATCC 29213 was very similar to those obtained with *S. aureus* ATCC 25923.

***E. faecalis* ATCC 29212 (Figure 3)**

- The pattern of zone diameters was similar to that obtained with the two isolates of *S. aureus.*
- All disks gave small zone diameters for Oxoid Isosensitest Agar (<16 mg/L Ca^{2+}).
- Inhibition zone diameters for BD, Rosco and Mast disks were considerably smaller (<14 mm) on Oxoid Isosensitest Agar than obtained on other media.

Etests

***S. aureus* ATCC 25923 (Figure 4)**

- The pattern of MICs associated with *S. aureus* ATCC 25923 was very to similar to those obtained with *S. aureus* ATCC 29213.

***S. aureus* ATCC 29213 (Figure 5)**

- The NCCLS expected QC range for MICs is 0.25 – 1 mg/L.
- Etests containing daptomycin alone gave satisfactory MICs on only two media, Mueller Hinton Agar manufactured by Remel and Hardy (32.6 mg/L and 27.1 mg/L Ca^{2+} respectively). The MICs were too high on BD Mueller Hinton Agar (~23 mg/L Ca^{2+}) and Oxoid Isosensitest Agar (<16 mg/L Ca^{2+}) and the MICs were too low on MAST Isotonic Agar (69.4 mg/L Ca^{2+}).
- Etests containing daptomycin and 50 mcg calcium gave satisfactory MICs on BD Mueller Hinton Agar (~23 mg/L Ca^{2+}) and Oxoid Isosensitest Agar (<16 mg/L Ca^{2+}) and low results on Remel and Hardy Mueller Hinton Agar (32.6 mg/L and 27.1 mg/L Ca^{2+} respectively) and MAST Isotonic Agar (69.4 mg/L Ca^{2+}).
- Etests containing daptomycin and 40 mcg calcium were only tested on Remel (32.6 mg/L Ca^{2+}) and BD Mueller Hinton Agar (21.3 mg/L Ca^{2+}) but gave satisfactory MICs on both media.

***E. faecalis* ATCC 29212 (Figure 6)**

- The NCCLS expected QC MIC range is 1 — 8 mg/L.
- Etests containing daptomycin alone gave satisfactory MICs on all media except Oxoid Isosensitest Agar (<16 mg/L Ca^{2+}).
- Etests containing daptomycin and 50 mcg calcium gave lower MICs than daptomycin alone but all were acceptable except those determined on Hardy Mueller Hinton Agar (27.1 mg/L Ca^{2+}) and Mast Isotonic Agar (69.4 mg/L Ca^{2+}).
- Disks containing daptomycin and 40 mcg calcium were only tested on Mueller Hinton Agar from BD (21.3 mg/L Ca^{2+}) and Remel (32.6 mg/L Ca^{2+}) but gave satisfactory MICs on both media.

CONCLUSIONS

- This study has demonstrated the importance of incorporating physiological levels of calcium in agar media to obtain accurate daptomycin MICs.
- Disks containing daptomycin alone from BD, Oxoid, and Mast resulted in acceptable QC values in range for all media except Oxoid Isosensitest Agar.
- With the exception of Mast Isotonic Agar, the addition of 100 mcg of calcium to daptomycin Mast disks and Rosco tablets provides for QC results within range for all media including Oxoid Isosensitest Agar (<16 mg/L Ca^{2+}).
- Etest containing daptomycin and 40 mcg of calcium provided optimal QC results using BD and Remel Mueller Hinton Agar.

REFERENCES

1. Koeth LM, Leclercq R, Olsson-Liljequist B. Comparison of daptomycin MIC results by DIN, NCCLS, SFM and SRGA methods for 297 Gram-positive organisms. International Journal of Antimicrobial Agents 23; 17-24. 2004.
2. National Committee for Clinical Laboratory Standards. Approved Standard M2-A6. Performance standards for antimicrobial disk susceptibility tests. 8th ed. NCCLS, Wayne, PA; 2003.