Daptomycin Disk Diffusion Testing: Comparison of Three Mueller Hinton Agars, Using Disks from Three Manufacturers

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Abstract

Background: Daptomycin, a cyclic lipopeptide antibiotic with good antimicrobial activity against most Gram-positive bacteria, was recently approved by the FDA. As the antimicrobial agent is not yet available for testing on most commercial MIC systems, disk diffusion testing will be the primary method of susceptibility testing initially. This study was performed in order to provide further validation of the disk diffusion susceptibility method using media and disks from the three primary suppliers in the U.S.A.

Methods: Three quality control strains (*S. aureus* ATCC 25923, S. aureus ATCC 29213 and *E. faecalis* ATCC 29212) were tested by disk diffusion. The first study tested each of the three strains once on the first day and five times on five additional days using BD and Hardy Mueller Hinton agar plates. The second study tested each of the strains five times for four days using a different lot of BD and Remel media. The 30 mcg. daptomycin disks were made by BD, Oxoid (Remel) and Mast (Hardy). Remel disks were included in the second study only. Calcium testing of each media was also performed using an ion selective electrode.

Results: Mean zone diameters and standard deviations (SD) for the NCCLS recommended disk quality control strain, S. aureus 25923 (expected daptomycin range 18-23 mm) were:

Daptomycin Disks	BD MHA Study 1 (n=26)		BD MHA Study 2 (n=20)		Remel MHA Study 2 (n=20)		Hardy MHA Study 1 (n=26)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BD	20.27	0.919	18.75	0.851	21.05	0.945	21.23	0.951
Remel	-	-	18.60	0.598	20.75	0.786	-	-
Hardy	19.42	0.857	17.50	0.688	19.40	1.984	20.23	0.765

Conclusion: Good correlation of daptomycin disk diffusion results was observed with disks from all three suppliers when tested with their respective MHA. The only occurrence of outlier results was with Hardy disks on one lot of BD media.

Introduction

Daptomycin is a lipopeptide antibiotic with a spectrum of activity against Gram-positive bacteria. The *in vitro* activity of daptomycin is influenced by the local concentration of calcium ions (Ca^{2+}) with maximum activity achieved in the presence of physiological levels of calcium ions 50 mg/L.

Consistency of susceptibility results using different media and antibiotic containing disks is important for providing clinicians with reliable information for antimicrobial prescribing and for accurate determination of resistance trends. Mueller Hinton agar (MHA) is the NCCLS recommended agar for disk diffusion testing of staphylococci, streptococci (supplemented with 5% lysed horse blood) and enterococci. There is, however, no recognized standard for calcium concentration in MHA. We studied the effect of the calcium ion concentration in three commercial agars distributed in the U.S. on the susceptibility test results using antibiotic disks containing 30 mcg daptomycin from three different manufacturers.

Materials and Methods

Media:

The following three commercial Mueller Hinton Agar prepared plates were tested in two studies:

- BBL (Becton Dickinson, Sparks, MD) – study 1 (batch nos. 3107327, 3107307) and study 2 (batch no. 395733)
- Hardy (Hardy Diagnostics, Santa Maria, CA) – study 1 (batch nos. 3106, 3132)
- Remel (Remel Inc., Lenexa, KA) – study 2 (batch no. 3282384)

Calcium Determination:

A sample of agar from a prepared plate was macerated, diluted 1:3 with sterile deionized water and allowed to sit overnight at 2-8°C. The water and agar mixture was centrifuged and supernatant removed and used for analysis. The free calcium, Ca^{2+}, concentration was performed using an ion selective electrode.[1]

Disks:

Thirty microgram daptomycin disks from each of the three different manufacturers:

- BBL (Becton Dickinson, Sparks, MD) – Studies 1 and 2
- Oxoid (Remel, Inc., Lenexa, KA) – Study 2
- Mast (Hardy Diagnostics, Santa Maria, CA) – Studies 1 and 2

QC strains:

Each reference strain was tested multiple times for 5 days in both studies.

S. aureus ATCC 25923

S. aureus ATCC 29213

E. faecalis ATCC 29212

Disk testing method: Zones of inhibition around disks were determined according to NCCLS guidelines for the different media, disk and QC strain combinations.

Results

Average Ca^{2+} concentration in media:

BD (Study 1): 23.9 mg/L

BD (Study 2): 21.3 mg/L

Remel: 32.6 mg/L

Hardy: 37.1 mg/L

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

- The NCCLS expected zone diameter range 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 Hardy disks was outside the expected range (17.50 mm mean, 17mm mode).
- Calculated 95% confidence limits based on +/- two standard deviations (SD) from mean zones were within the expected range of 18-23 mm for all disks on Remel and Hardy media.
- Range of zone diameters was 3-5 mm for all disk and media combinations. Most reproducible results (zone range of 3 mm) were obtained with Remel disks on BD MHA (Study 2).

***S. aureus* ATCC 29213 (Figure 2, Table 1)**

- 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.
- Range of zone diameters was 2-5 mm for all disk and media combinations. Most reproducible results (zone range of 2 mm) were obtained with Remel disks on BD MHA (Study 2) and Hardy disks on Remel MHA.

***E. faecalis* ATCC 29212 (Figure 3, Table 1)**

- The pattern of zone diameters was similar to that obtained with the two isolates of *S. aureus*.
- Smallest zones (14.75 mm mean; 15 mm mode) were seen with Hardy disks on BD-2 media.
- Range of zone diameters was 3-4 mm for all disk and media combinations. Most reproducible results (zone range of 3 mm) were obtained with BD disks on BD (Study 2) MHA and Hardy disks on BD (Study 1) MHA and Remel MHA.

Conclusions

- Smallest zone diameters for all three strains were obtained using media with the lowest calcium level (BD MHA from Study 2) and largest zone diameters were obtained with media with the highest calcium level (Hardy).
- Acceptable results with *S. aureus* 25923 were obtained with media containing 23.9 to 37.1 mg/L Ca^{2+}
- Difference of 1 mm observed with Hardy disks was only significant with media containing lowest level of Ca^{2+} (BD MHA from Study 2).
- Quality control strain, *S. aureus* 25923, is a good indicator of adequate Ca^{2+} levels in MHA for accurate disk diffusion testing of daptomycin.

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. *Int J 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; **2004**

Table 1: Summary of disk results for all media and disk manufacturers (mm)

MHA	BD (BBL) Disks Mean	SD	Mode	Min-Max	Remel (Oxoid) Disks Mean	SD	Mode	Min-Max	Hardy (Mast) Disks Mean	SD	Mode	Min-Max
S. aureus 25923												
BD-1	20.19	0.931	20	18-22	na	na	na	na	19.25	0.950	20	17-21
BD-2	18.75	0.851	18	18-21	18.60	0.598	19	18-20	17.50	0.688	17	16-19
Remel	21.05	0.945	20	20-23	20.75	0.786	21	19-22	19.90	0.912	20	18-22
Hardy	21.09	0.928	21	20-23	na	na	na	na	20.03	0.801	20	19-22
S. aureus 29213												
BD-1	19.50	1.078	19	17-21	na	na	na	na	18.38	1.008	18	17-20
BD-2	19.35	0.587	19	18-20	19.40	0.503	19	19-20	17.65	0.745	17	17-20
Remel	20.65	0.671	21	20-22	20.55	0.686	20	20-22	19.40	0.503	19	19-20
Hardy	21.25	0.916	21	20-23	na	na	na	na	20.34	1.066	20	18-22
E. faecalis 29212												
BD-1	17.81	0.801	18	16-19	na	na	na	na	16.38	0.697	17	15-17
BD-2	16.70	0.571	17	16-18	15.55	0.686	16	14-17	14.75	0.967	15	13-16
Remel	18.20	0.951	19	17-20	17.50	0.827	17	16-19	16.35	0.671	16	15-17
Hardy	19.62	0.898	20	17-21	na	na	na	na	18.35	0.846	19	17-20

Figure 1. Mean zone diameter (mm) and upper and lower limits of 95% confidence limits (+/- 2 SD) for *S. aureus* ATCC 25923

(Red Box indicates NCCLS approved QC range of 18-23 mm)

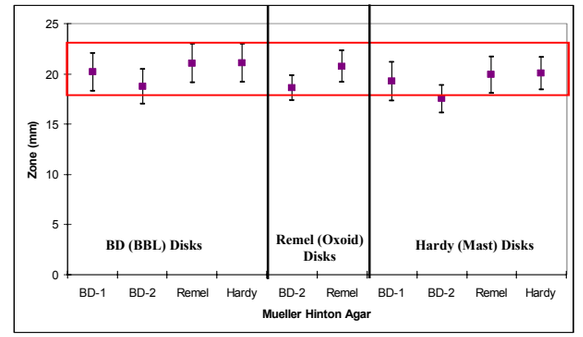

Figure 2. Mean zones (mm) and upper and lower limits of 95% confidence limits (+/- 2 SD) for *S. aureus* ATCC 29213

No NCCLS QC range for S. aureus 29213

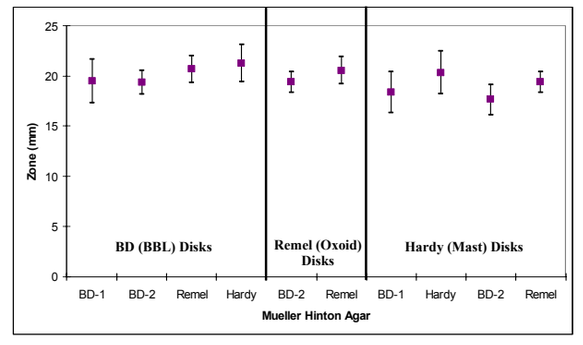

Figure 3. Mean zones (mm) and upper and lower limits of 95% confidence limits (+/- 2 SD) for *E. faecalis* ATCC 29212

No NCCLS QC range for E. faecalis 29212