

Evaluation of daptomycin 5, 10 and 15 mcg discs on IsoSensitest and Mueller Hinton agar against *Staphylococcus aureus*

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Abstract (revised)

Objective: This study was performed in order to determine if 5, 10 or 15 mcg daptomycin discs could be used reliably during disc diffusion for the detection of susceptible and non-susceptible *S. aureus* on either IsoSensitest agar or Mueller Hinton agar.

Methods: Eight daptomycin/calcium discs at concentrations of 5/50, 10/50, 15/10, 15/50, 5/25, 10/25, 15/5 and 15/25 mcg were tested on 2 lots of IsoSensitest agar (ISA1 and ISA2) using semi-confluent growth according to BSAC and SRGA methods. Three daptomycin discs (2 manufacturers) at concentrations of 5, 10 and 15 mcg were tested on 2 lots of Mueller Hinton agar (MHA1 and MHA2). All discs were tested against 39 stock strains (including a challenge set consisting of 15 strains with daptomycin MIC=2, 12=4 and 1=8 mcg/mL) and 49 clinical strains of *S. aureus* in 2 laboratories. The QC strain, *S. aureus* ATCC 25923 was tested on each day of testing.

Results: A non-susceptible breakpoint (NS BP) was chosen for each of the disc configurations based on analysis of susceptible and non-susceptible zone sizes in order to limit very major errors (VME). All 12 strains with daptomycin MICs of 4 mcg/mL were reliably detected by all discs. The number of clinical (susceptible) strains that were categorized as non-susceptible and the number of stock strains with MICs of 2 mcg/mL (n=13) that were categorized as susceptible were:

Disc	NS BP	No. of clinical strains = NS				No. of stock strains MIC of 2 = S			
		BD discs		Mast discs		BD discs		Mast discs	
		MHA1	MHA2	MHA1	MHA2	MHA1	MHA2	MHA1	MHA2
5	≤13	0	0	6	3	0	0	0	0
10	≤15	1	0	6	11	1	1	0	0
15	≤17	1	7	21	34	0	1	0	0

Disc	NS BP	Oxoid discs		Mast discs		Oxoid discs		Mast discs	
		ISA1	ISA2	ISA1	ISA2	ISA1	ISA2	ISA1	ISA2
5/50	≤14	—	—	22	35	—	—	0	0
10/50	≤15	—	—	13	10	—	—	0	1
15/10	≤13	—	—	12	18	—	—	0	0
15/50	≤16	—	—	48	57	—	—	2	0
5/25	≤12	1	1	—	—	3	3	—	—
10/25	≤14	2	1	—	—	1	1	—	—
15/5	≤10	2	2	—	—	0	0	—	—
15/25	≤14	4	3	—	—	2	1	—	—

Conclusions: Daptomycin 5 mcg discs on MHA and daptomycin/calcium 15/5 on ISA were best methods for detection of non-susceptible strains with MICs of 2 and in detection of susceptible strains. BD daptomycin discs on MHA were less likely to result in categorization of susceptible strains as non-susceptible compared to Mast discs, although Mast discs were slightly better at detection of non-susceptible strains compared to BD discs. Further assessment utilizing multiple test sites, disc and media batches is necessary for method validation.

Introduction

It has been shown that disc testing using the daptomycin 30 mg discs with small amounts of calcium (5, 10, 15 and 20 µg) on IsoSensitest agar adequately distinguishes non-susceptible from susceptible *Staphylococcus aureus* (see poster 1442; Koeth *et al.*). The present study was performed in order to further validate the BSAC/SRGA procedure, to evaluate the effects of discs with lower levels of daptomycin at varying levels of calcium on different lots of IsoSensitest agar, to additionally evaluate daptomycin only discs on Mueller Hinton Agar and to determine any variations due to different laboratories and disc manufacturers.

Methods

Testing laboratories

City Hospital, Birmingham, UK
Central Hospital, Växjö, Sweden

Microorganisms

- 49 recently isolated *S. aureus* collected from each of the two testing sites.
- 39 stock strains (includes recent pre and post-treatment staphylococci pairs and additional non-susceptible strains with daptomycin MICs of 2 and 4 mg/L.
- S. aureus* ATCC 25923.

Media

Each lab tested 2 lots of Mueller Hinton agar (MHA) and 2 lots of IsoSensitest agar (ISA). One lot of each was a common lot tested by both laboratories.

Disc testing methodology

BSAC/SRGA disc method was utilized, which includes:

- Plate inoculation with organism suspension equivalent to 0.5 McFarland and diluted 1:10 (semi-confluent growth).
- Plate incubation at 35–37°C in air for 18–20 hours.

Antimicrobial discs

Tested on ISA				Tested on MHA			
Daptomycin/calcium (mcg)	Disc supplier	Daptomycin (mcg)	Disc supplier	Daptomycin (mcg)	Disc supplier	Daptomycin (mcg)	Disc supplier
5/50	Mast	5	BD and Mast	5	BD and Mast	5	BD and Mast
10/50	Mast	10	BD and Mast	10	BD and Mast	10	BD and Mast
15/10	Mast	15	BD and Mast	15	BD and Mast	15	BD and Mast
15/50	Mast						
5/25	Oxoid						
10/25	Oxoid						
15/5	Oxoid						
15/25	Oxoid						

Calcium analysis of agar

All lots of agar were tested for calcium concentration by Laboratory Specialists, Inc., Westlake, OH using ion selective electrode methodology.

Results

ISA

Zone results for each of the discs by testing site and strain type (stock or clinical) are summarized in Table 1.

- Discs with 50 mcg of calcium were excellent at detecting non-susceptible strains, however, there was a very high percentage of major errors (ME) (susceptible strains that were classified as non-susceptible).
- There was minimal lab to lab variation. Zone diameters from the Swedish lab tended to be 1 mm larger than the UK lab and as a consequence, there was a slightly higher rate of false susceptible results in Sweden.
- No lot to lot variation was observed between testing media (Table 3).
- Oxoid 15/5 disc produced no very major errors (VME) (non-susceptible strains that were classified as susceptible) and the least number of ME in both testing sites. False resistant rates were 4.8% for stock/challenge strains and 2.6% for clinical strains. A scatterplot of all data is shown in Figure 1.

MHA

Zone results for each of the discs by disc manufacturer, testing site and strain type (stock or clinical) are summarized in Table 2.

- The largest number of ME occurred with the 15 mcg disc.
- Lab to lab variation, although only approximately 1 mm larger in the Swedish lab, was evident based on the assessment of ME. There were larger numbers of ME for both stock and clinical strains at the UK site. This difference was most prevalent with the Mast discs.
- No lot to lot variation was observed between media (Table 4).
- Both the BD and Mast 5 disc were superior at detecting non-susceptible isolates – there were no VME at either testing site. UK ME rates were 16.3% for stock/challenge strains and 3.6% for clinical strains. Swedish ME rates were 1.9% for stock/challenge strains and 1.0% for clinical strains. A scatterplot of all data is shown in Figure 2.

Table 3. ISA – Mean zone diameters (mm) of stock/challenge strains by media lot and testing site.

Disc (µg) Dap/Ca	UK tested (n=38)		Sweden tested (n=39)	
	Common lot (Oxoid #409732) [Ca++]=11.1 mg/L	Site specific lot (Oxoid #372715) [Ca++]=9.0 mg/L	Common lot (Oxoid #409732) [Ca++]=11.1 mg/L	Site specific lot (Oxoid #388520) [Ca++]=8.8 mg/L
Mast:				
5/50	12.2	12.1	12.8	12.5
10/50	13.8	13.8	14.4	14.2
15/10	10.9	10.8	11.6	11.0
15/50	14.5	14.4	15.9	15.5
Oxoid:				
5/25	10.1	10.0	11.1	11.2
10/25	11.6	11.5	12.5	12.8
15/5	9.2	8.5	9.5	9.3
15/25	12.6	12.5	13.6	13.8

Table 4. MHA – Mean zone diameters (mm) of stock/challenge strains by media lot and testing site.

Disc (µg) BD & Mast	UK tested (n=78)		Sweden tested (n=78)	
	Common lot (Oxoid lot #414523) Ca=26.0 mg/L	Site specific lot (Lot #389630) Ca=24.2 mg/L	Common lot (Oxoid lot #414523) Ca=26.0 mg/L	Site specific lot (Lot #381749) Ca=25.6 mg/L
5	10.2	10.0	10.9	11.2
10	12.9	12.6	13.5	13.8
15	14.6	14.2	15.2	15.4

Table 1. ISA – Summary of zone diameters and number of categorical errors for stock and clinical strains for each disc type by testing site.

Disc (DAP/Ca mcg)	S/R BP	Site	Challenge strains								Clinical strains ⁴			
			Zone (mm)				Zone (mm)				VME	Number of: ME	Zone (mm) Range	Number of: ME
			AIP ¹ isolates		NS ² isolates		S ³ isolates		S ³ isolates					
Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean					
Mast discs:														
5.0/50	14	UK Sweden	9 to 15	12.2	9 to 14	11.1	13 to 16	14	0	17	13 to 17	51		
			9 to 16	12.6	9 to 14	11.4	14 to 16	15.2	0	3	14 to 28	6		
10.0/50	15	UK Sweden	10 to 17	13.8	10 to 15	12.7	14 to 17	15.6	0	7	15 to 19	19		
			11 to 18	14.3	11 to 15	13.2	15 to 18	16.6	1	1	14 to 19	5		
15.0/10	13	UK Sweden	6 to 14	10.8	6 to 12	9.6	12 to 14	13.0	0	17	12 to 16	25		
			7 to 16	11.3	7 to 13	9.9	12 to 16	13.9	0	7	13 to 18	5		
15.0/50	17	UK Sweden	11 to 18	14.5	11 to 17	13.5	15 to 18	15.9	0	22	15 to 20	69		
			12 to 19	15.7	12 to 18	14.7	17 to 19	17.8	2	10	15 to 21	36		
Oxoid discs:														
5.0/25	11	UK Sweden	6 to 13	10.0	6 to 12	8.9	11 to 13	12.3	1	4	11 to 14	2		
			8 to 14	11.1	8 to 13	10.0	12 to 14	13.5	5	0	13 to 15	0		
10.0/25	13	UK Sweden	8 to 15	11.5	8 to 13	10.4	12 to 15	13.8	0	9	13 to 16	3		
			10 to 16	12.7	10 to 14	11.4	13 to 16	15.2	2	1	14 to 17	0		
15.0/5	10	UK Sweden	6 to 15	8.8	6 to 10	7.6	8 to 15	11.2	0	5	9 to 13	5		
			6 to 13	9.4	6 to 10	8.2	10 to 13	11.8	0	3	11 to 14	0		
15.0/25	14	UK Sweden	8 to 16	12.6	8 to 14	11.4	10 to 16	14.8	0	5	14 to 17	7		
			10 to 17	13.7	10 to 15	12.5	15 to 17	16.2	3	0	15 to 19	0		

¹AIP isolates – UK: n=76 (38 strains x 2 media lots); Sweden: n=78 (39 strains x 2 media lots). ²NS (non-susceptible isolates (MICs >1 mcg/mL)) – UK: n=50 (25 strains x 2 media lots); Sweden: n=52 (26 strains x 2 media lots). ³S – [susceptible isolates (MICs ≤1 mcg/mL)] – n=26 (13 strains x 2 media lots). ⁴UK: n=96 (48 strains x 2 media lots); Sweden: n=98 (49 strains x 2 media lots).

Table 2. MHA – Summary of zone diameters and number of categorical errors for stock and clinical strains for each disc type by testing site.

Disc (DAP mcg)	S/R BP	Site	Challenge strains								Clinical strains ⁴			
			Zone (mm)				Zone (mm)				VME	Number of: ME	Zone (mm) Range	Number of: ME
			AIP ¹ isolates		NS ² isolates		S ³ isolates		S ³ isolates					
Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean					
BD discs:														
5	13	UK Sweden	6 to 15	10.6	6 to 13	8.6	13 to 16	14.6	0	5	14 to 17	0		
			6 to 17	11.5	6 to 13	9.4	14 to 17	15.5	0	0	15 to 21	0		
10	15	UK Sweden	8 to 19	13.3	8 to 15	11.5	15 to 19	16.9	0	1	16 to 20	0		
			8 to 19	13.9	8 to 16	12.1	16 to 19	17.6	1	0	17 to 22	0		
15	17	UK Sweden	10 to 20	14.7	10 to 17	13.1	16 to 20	18.0	0	8	16 to 21	8		
			8 to 20	15.9	8 to 18	14.3	17 to 20	19.0	1	1	18 to 22	0		
Mast discs:														
5	13	UK Sweden	6 to 15	9.6	6 to 12	7.5	12 to 15	13.5	0	12	12 to 18	7		
			6 to 16	10.6	6 to 13	8.5	12 to 16	14.8	0	2	12 to 19	2		
10	15	UK Sweden	6 to 18	12.2	6 to 15	10.3	13 to 18	15.8	0	10	14 to 18	17		
			8 to 19	13.4	8 to 15	11.4	16 to 19	17.3	0	0	17 to 22	0		
15	17	UK Sweden	8 to 20	14.0	8 to 17	12.3	15 to 20	17.3	0	17	13 to 21	49		
			9 to 20	14.8	9 to 17	13.0	16 to 20	18.3	0	2	17 to 21	6		
Mast and BD discs combined:														
5	13	UK Sweden	6 to 16	10.1	6 to 13	8.1	12 to 16	14.1	0	17	12 to 17	7		
			6 to 17	11.1	6 to 13	9.0	12 to 17	15.2	0	2	12 to 21	2		
10	15	UK Sweden	6 to 19	12.7	6 to 15	10.9	13 to 19	16.4	0	11	14 to 20	17		
			8 to 19	13.6	8 to 16	11.8	16 to 19	17.4	1	0	17 to 22	0		
15	17	UK Sweden	8 to 20	14.4	8 to 17	12.7	15 to 20	17.7	0	25	13 to 21	57		
			8 to 20	15.3	8 to 18	13.7	16 to 20	18.6	1	3	17 to 22	6		

¹AIP isolates – n=156 (39 strains x 2 discs x 2 media lots). ²NS (non susceptible isolates (MICs >1 mcg/mL)) – n=104 (26 strains x 2 discs x 2 media lots). ³S – [susceptible isolates (MICs ≤1 mcg/mL)] – n=52 (13 strains x 2 discs x 2 media lots). ⁴n=196 (49 strains x 2 discs x 2 media lots).

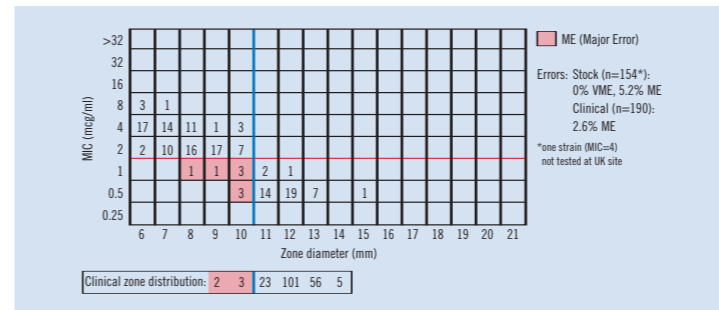


Figure 1. Daptomycin 15 mcg/Calcium 5 mcg disc on ISA: Number of stock isolates at each MIC and disc zone (Oxoid discs x 2 ISA lots x 2 sites).

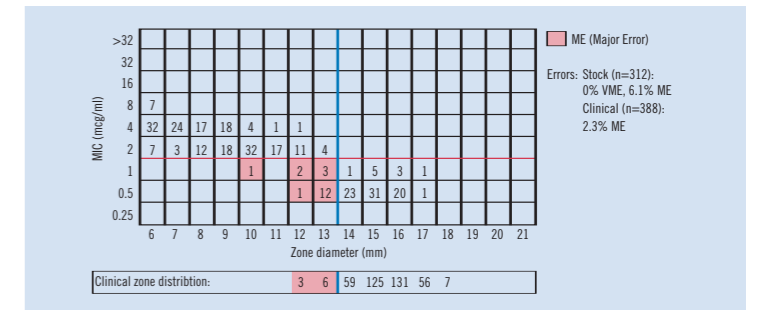


Figure 2. Daptomycin 5 mcg disc on MHA: Number of stock isolates at each MIC and disc zone (BD and Mast discs x 2 MHA lots x 2 sites).

CONCLUSIONS

- The Oxoid 15/5 disc on ISA agar was superior to the other discs tested. All non-susceptible strains were detected with a minimal level of ME.
- The BD 5 and BD 10 discs on MHA were very similar in performance. The BD 5 disc detected all non-susceptible strains and there was one strain undetected at the Swedish site using the BD10 disc. Both discs had low rates of ME.
- A wide separation of zones between susceptible and non-susceptible strains is desired, although not achieved with any of the discs and media tested. Therefore, a higher rate of ME are expected in order to prevent occurrence of VME.
- Further assessment at multiple labs and using discs from different manufacturers and agar lots is required in order to further validate the methods.