

Evaluation of effect of low concentrations of calcium in daptomycin 30 mcg discs on IsoSensitest agar against staphylococcus.

Laura Koeth¹, Robert Smyth², Gunnar Kahlmeter².

¹Laboratory Specialists, Westlake, Ohio; ²Central Hospital, Växjö, Sweden

Abstract

Objectives: Daptomycin disc diffusion testing using the 30 mg disc and Mueller Hinton agar is not correctly recommended by CLSI due to an inability to detect some *S. aureus* isolates. Levels of ionized calcium in Mueller Hinton agar have been variable between manufacturers and batches, ranging from 17–64 mg/L. In contrast, calcium levels in IsoSensitest agar have been found to be more consistent and at lower levels of approximately 10 mcg/L. This study was performed as an initial screen to evaluate daptomycin 30 mcg discs containing calcium between 0 and 20 mcg on IsoSensitest agar as a potential disc method.

Methods: Daptomycin 30 mcg discs containing 5, 10, 15 and 20 mcg of calcium were utilized against a challenge set of *S. aureus* with elevated daptomycin MICs and against a set of recently isolated daptomycin susceptible *S. aureus*. The QC strains, *S. aureus* ATCC 25923 and *E. faecalis* 29212 were tested on each day of testing.

Results: Zone diameter ranges for each of the discs were:

Challenge <i>S. aureus</i> by daptomycin (mcg/mL)	Calcium concentration (mcg) of daptomycin 30 mcg disc							
	20	15	10	5	20	15	10	5
1 (n=17)	13–16	0	11–15	6	11–15	11	9–14	7
2 (n=23)	11–17	1	10–15	2	10–15	4	8–14	3
4 (n=14)	10–14	0	9–12	0	8–12	0	7–11	0
4, 8 (n=3)	11, 16	0	9, 10	0	9, 9	0	8, 8	0
Recent clinical strains	Range (mm)	Range (mm)	Range (mm)	Range (mm)	Range (mm)	Range (mm)	Range (mm)	Range (mm)
<i>S. aureus</i> (n=77)	17–20	15–18	13–18	13–18				
All staphylococci (n=177)	17–25	15–24	13–24	13–24				

Conclusions: The daptomycin 30 mcg disc with 5, 10, 15 or 20 mcg of calcium when used with IsoSensitest agar could potentially detect all strains with MICs of ≥ 4 mcg/mL. The 20 mcg calcium disc was the best disc for detection of non-susceptible strains with MICs = 2 mcg/mL. However, the 10 mcg calcium disc was superior at detection of susceptible strains with MICs of 1. The introduction of a buffer zone could resolve some discrepancies observed at MICs of 1 and/or 2 mcg/mL. Additional testing of lower daptomycin concentration discs with similar levels of calcium may provide further separation of zones between susceptible and non-susceptible strains.

Introduction

In prior studies, very major errors occurred in the comparison of daptomycin MIC to daptomycin discs using Mueller Hinton agar and calcium supplemented IsoSensitest agar. However, these non-susceptible strains did test non-susceptible using agar with low levels of calcium (i.e. non-supplemented IsoSensitest agar). Zone diameters increased beyond the susceptible breakpoint with as little as 20 mcg of calcium added to the disc. This study was performed to determine if a level of calcium between 0 and 20 mcg, is an appropriate amount of calcium to use for the detection of non-susceptible strains.

Methods

Testing laboratories

Central Hospital, Växjö, Sweden

Microorganisms

- 177 recently isolated *Staphylococci* collected from testing site (77 *S. aureus* and 100 coagulase negative staphylococci)
- 57 stock challenge *S. aureus*, with MICs (as determined previously by CLSI microbroth dilution) as follows:

Number	MIC (mcg/mL)
17	1
23	2
14	4
3	8

- S. aureus* ATCC 25923

Media

1 lot IsoSensitest agar (ISA – Oxoid, Basingstoke, UK), plates prepared by testing site.

Disc testing methodology

BSAC/SRGA disc method was utilized, which include:

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

Quality control strain was setup every test day

Antimicrobial discs (Mast, Bottle UK):

Daptomycin/calcium (mcg)
30
30/5
30/10
30/15
30/20

Calcium analysis of agar

A sample of IsoSensitest agar (ISA) was sent to Laboratory Specialists, Inc. (LSI, Westlake, OH) for analysis of free calcium content utilizing ion electrode methodology. The agar was macerated and diluted 1:3 with sterile distilled water and allowed to sit overnight at 2–8°C. The water and agar mixture was centrifuged and supernatant removed and used for calcium analysis.

Results

Mean zone results for each of the discs by strain type (non-susceptible stock [MICs >1] and clinical) are shown in Table 1 and Figure 1. Correlation of zone and MIC results for each of the discs is depicted in scatter plots in Figures 2 and 3.

- Ionized calcium level of IsoSensitest agar was 10.1 mcg/mL
- Mean zone difference between non-susceptible challenge strains and clinical strains is approximately 5 mms for all disc types
- Zone diameters decrease linearly with decreasing levels of calcium
- If susceptible breakpoint is set to the lowest clinical strain zone, all challenge strains with MICs of 4 and 8 are accurately categorized as resistant and some challenge strains with MICs of 2 would result in very major errors (5.3% with Dap30/5, 7.0% with Dap30/10, 3.5% with Dap30/15 and 1.7% with Dap30/20)
- If susceptible breakpoint is moved to eliminate all very major errors, all challenge strains with MICs = 1 mcg/mL and a small percentage of clinical strains (4.5% with Dap30/Ca5 and Dap30/Ca10, 0.6% with Dap30/Ca15 and 15.3% with Dap30/Ca20) would result in major errors

Table 1. Mean zone diameters (mms) of *S. aureus* with discs containing daptomycin (30 mcg) and variable calcium (5–20 mcg) when tested on IsoSensitest agar.

	DAP30/Ca++20	DAP30/Ca++15	DAP30/Ca++10	DAP30/Ca++5	DAP30
Clinical strains (n=77)	17.86	16.83	16.22	15.39	14.29
Non-susceptible stock strains (n=43)	13.33	11.71	11.1	10	8.6
Mean difference between clinical and stock	4.53	5.12	5.12	5.39	5.69

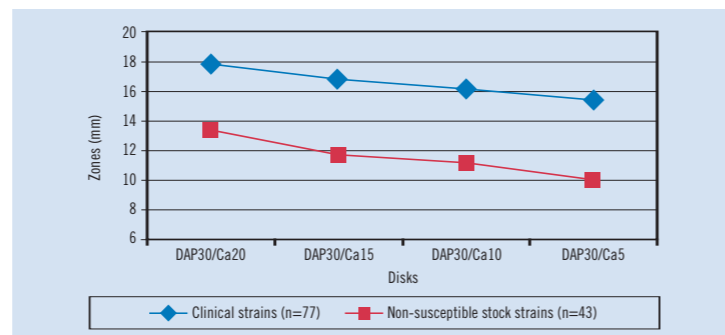


Figure 1. Mean zone diameters (mms) of *S. aureus* with discs containing daptomycin (30 mcg) and variable calcium (5–20 mcg) when tested on IsoSensitest agar.

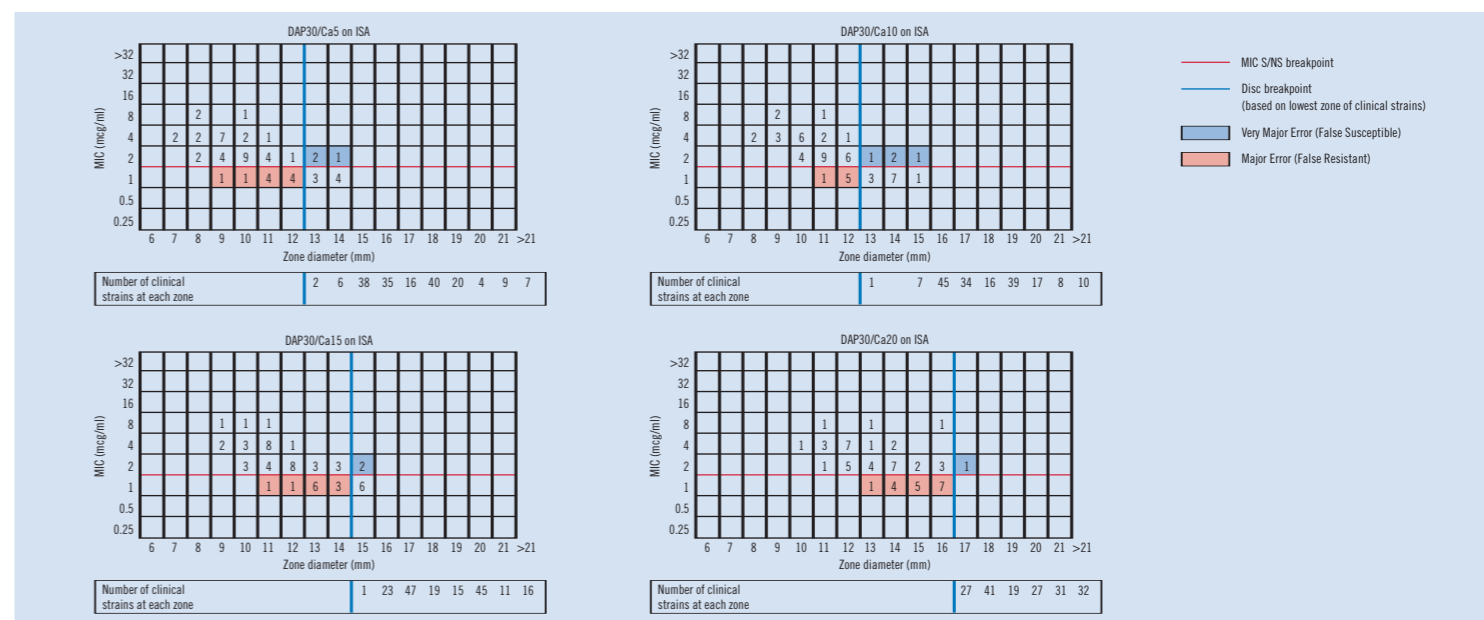


Figure 2. MIC and disc scatter plots of challenge *S. aureus* (n=57) and MIC frequency distributions of clinical staphylococci (n=177) by disc. Susceptible/non-susceptible breakpoint based on lowest zone of clinical strain distribution.

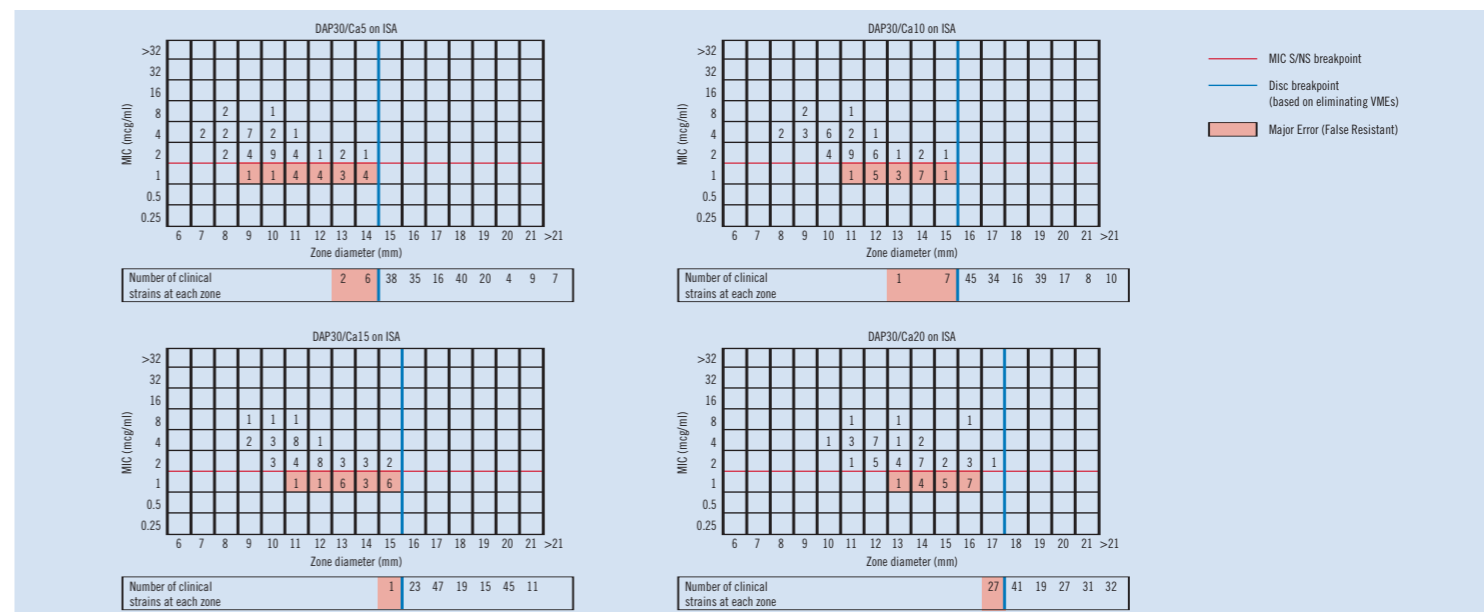


Figure 3. MIC and disc scatter plots of challenge *S. aureus* (n=57) and MIC frequency distributions of clinical staphylococci (n=177) by disc. Susceptible/non-susceptible breakpoint based on eliminating very major errors (VMEs).

CONCLUSIONS

- Because of the relatively narrow range of zones and in order to reduce likelihood of a very major error, susceptible strains with MICs of 1 mcg/mL would have a high probability of being categorized as non-susceptible with any of the discs tested. Clinical isolates with MICs = 1 mcg/mL are currently not encountered frequently
- MICs for the clinical strains were not determined, however, the majority would be categorized as susceptible with any of the discs tested. Assuming all clinical isolate MICs are in the susceptible range, the highest level of false resistance was observed with the Dap30/Ca20 disc
- When the breakpoint is moved to eliminate all very major errors, the Dap30/Ca15 disc provided the least number of major errors
- Further assessment at multiple labs and using discs from different manufacturers and agar lots is required in order to further validate the methods
- Further testing of discs with lower levels of daptomycin and similar levels of calcium may provide a better separation of zones between susceptible and non-susceptible strains (see poster 1441; Koeth et al.)

References

Sader HS, Fritsche TR, Jones RN. (2003) *Diagn Microbiol Infect Dis* 2005; 53: 329–32. Antimicrobial activity of daptomycin against clinical strains of indicated species isolated in North American medical centers.
 Sader HS, Fritsche TR, Jones RN. Update on daptomycin antimicrobial activity when tested against clinical strains from North American Medical Centers (2004) IDSA 2005, Poster 443.