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Comparison of Daptomycin and Vancomycin MICs for 244 Gram-positive Strains Using IsoSensitest and Mueller Hinton Media

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Revised Abstract

Objective: To determine the correlation between MIC results obtained with IsoSensitest Agar and Mueller Hinton Broth for selected Gram-positive organisms.

Methods: Daptomycin (DAP) and vancomycin (VAN) MICs for 244 pathogens including stock and fresh clinical isolates of enterococci, staphylococci, and streptococci were determined by SRGA agar dilution methodology using IsoSensitest (ISO) agar and by NCCLS broth microdilution methodology using Mueller Hinton (MH) broth. Both media were supplemented with calcium so that final calcium concentration was 50 mg/L. Quality control strains were tested on each day.

Results: Essential Agreement Rates (EA% +/– 1 doubling dilution) of ISO compared to MH MICs were:

Organism	Essential Agreement % DAP ISO	VAN ISO
E faecium (n=30)	96.7	100
E faecalis (n=30)	92.9	100
S aureus (n=50)	98.0	100
S epidermidis (n=48)	89.4	100
Viridans strep (n=36)	100	100
S agalactiae (n=25)	100	100
S pyogenes (n=25)	96.0	96.0

Both vancomycin and daptomycin ISO MICs tended to be slightly lower than MH MICs, although the difference was greater with daptomycin.

Conclusions: Overall, there was good correlation of daptomycin ISO and MH MICs.

Introduction

A variety of MIC procedures are used to assess an antimicrobial agent's *in vitro* activity. IsoSensitest media is the preferred media for some of the susceptibility methods in Europe. Mueller Hinton media is the recommended media of the National Committee of Clinical Laboratory Standards (NCCLS) reference procedure. In this study, the *in vitro* activity of daptomycin to 244 Gram-positive isolates was assessed by the NCCLS microdilution method (calcium supplemented Mueller Hinton Broth) and by the Swedish Reference Group for Antibiotics (SRGA) agar dilution methods (calcium supplemented IsoSensitest Agar). As daptomycin requires the presence of physiological concentrations of calcium ions for optimal antibiotic activity, the free calcium concentration in the test medium is an important issue with regard to MIC results obtained with various MIC testing methods. This study, therefore, included calcium supplementation of the media and calcium assay of the medium prior to testing in order to achieve optimal calcium ion levels of 50 mg/L.

Materials and Methods

Antimicrobial Agents

Daptomycin at concentrations of 0.008 to 16 µg/mL and vancomycin at concentrations of 0.06 to 128 µg/mL were tested by NCCLS microdilution method using Sensititre Plates (Trek Diagnostics, E. Grinstead, UK). The dilutions tested by organism species for SRGA agar dilution methods were:

Organism	Vancomycin MIC Range (µg/mL)	Vancomycin No. of Dilutions	Daptomycin MIC Range (µg/mL)	Daptomycin No. of Dilutions
Staphylococcus aureus	0.06-16	9	0.03-16	10
Coagulase negative Staphylococcus	0.06-16	9	0.03-16	10
Enterococcus faecium	0.12-128	11	0.03-32	11
Enterococcus faecalis	0.12-128	11	0.03-32	11
Streptococcus pyogenes	0.06-4	7	0.03-4	8
Streptococcus agalactiae	0.06-4	7	0.03-4	8
Viridans group streptococci	0.06-4	7	0.03-4	8

Isolates

One half of the strains were stock isolates provided by Laboratory Specialists, Inc. (LSI) and the other half were clinical isolates collected within the last year from Central Hospital, Växjö Sweden The following organisms were tested: *E faecium*, *E faecalis*, *S aureus*, *S epidermidis*, viridans group streptococci, *S agalactiae* and *S pyogenes*.

Methods

Testing Sites

MIC Testing – Central Hospital, Växjö, Sweden
Calcium Testing – Laboratory Specialists, Inc.

Testing of Calcium Content of Media

1. The agar was macerated and diluted 1:3 with sterile distilled water and allowed to sit overnight at 2-8°C.
2. The water and agar mixture was centrifuged and supernatant removed and used for calcium analysis.
3. Testing was performed using ion electrode methodology.

After testing of different amounts of calcium supplementation, it was determined that a 1:100 dilution of a 0.1M $CaCl_2$ solution (40 µg/mL calcium) in IsoSensitest Agar would provide a final optimum free calcium concentration of 50 µg/mL. LSI repeated calcium analysis in prepared plates to confirm final calcium concentration.

MIC Testing Procedures

NCCLS microdilution (NCCLS CSMHB):*

**Daptomycin wells in the Sensititre MIC trays contain calcium so that final calcium concentraton after addition of cation adjusted Mueller Hinton broth (CAMHB) is 50 µg/mL, which is equivalent to calcium-supplemented Mueller Hinton broth (CSMHB).*

1. Inocula were prepared from blood agar plates incubated for 18-20 hours by the following direct colony suspension method.
2. An organism suspension equivalent to a density of a 0.5 McFarland standard (10^8 cfu/mL) was made in a tube of CAMHB. 50 µL was transferred to 11 ml CAMHB broth tubes for staphylococci and enterococci testing. 200 µL was transferred to 10 mL CAMHB + 5% Lysed Horse Blood for streptococci testing
3. 100 µL of organisms suspension was dispensed into each well of the MIC tray.
4. Trays were incubated under ambient conditions at 35°C for 24 hours and lowest drug concentration showing no growth was read as the MIC.

SRGA agar dilution (SRGA CSISA)

The base medium was IsoSensitest Agar (Oxoid, England) with calcium chloride supplementation for staphylococci and enterococci testing. IsoSensitest calcium supplemented agar supplemented with 5% defibrinated horse blood for streptococci testing.

1. Antibiotic dilutions were prepared, and 0.5 mL added to each 100 mL molten agar.
2. Inocula were prepared from blood agar plates incubated for 18-20 hours by the direct colony suspension method. An organism suspension equivalent to a density of a 0.5 McFarland standard (10^8 cfu/mL) was made in a tube of phosphate buffered saline (PBS). 100 µL was transferred to 10 mL PBS and this suspension was used as inoculum.
3. A 19-pin inoculator, (Mast Diagnostics, England) delivering 1-2 µL per pin, was used to apply the suspensions to the agar surface. All plates were allowed to dry at room temperature before incubation at 36 ± 1°C for 18 ± 2 hours (in ambient conditions for staphylococci and enterococci and in an atmosphere of 5 ± 1% CO_2 in air for streptococci.

Quality Control:

S aureus (ATCC 29213) and *E faecalis* (ATCC 29212) were tested on each day of staphylococci and enterococci testing and *Streptococcus pneumoniae* (ATCC 49619) was tested on each day of streptococci testing. Results for the study isolates were only acceptable if quality control results were within NCCLS ranges.

Results

1. The calcium content of the ISA before supplementation was 12.3 µg/mL. After 40 µg/mL of calcium was added to the agar, the calcium concentration was 49.08 µg/mL.
2. MIC results by NCCLS MHB and SRGA ISA methods are summarized in Tables 1 and 2 and Figure 1.
3. Overall, for all study isolates, 95.5% of SRGA daptomycin MIC results were within +/– 1 doubling dilution from the NCCLS MIC results. In comparison, essential agreement rates for vancomycin by individual species were 100%, with the exception of *S pyogenes* which was 96%. Daptomycin essential agreement rates were 95% or greater for all species except *E faecalis* (92.9%) and *S epidermidis* (89.6%). SRGA MICs for both *E faecalis* and *S epidermidis* tended to be lower than NCCLS (approximately 68% were 1 doubling dilution lower). Of the 28 *E faecalis*, 1 strain had SRGA daptomycin MICs 2 doubling dilutions lower than NCCLS and 1 strain had SRGA daptomycin MICs 3 doubling dilutions lower than NCCLS. Of 48 *S epidermidis*, 5 strains had SRGA daptomycin MICs 2 doubling dilutions lower than NCCLS. Other SRGA MICs that differed by greater than +/– 1 doubling dilution were 1 *E faecium* that was 2 doubling dilutions lower by SRGA and 1 *S pyogenes* that was 4 doubling dilutions lower by SRGA. All quality control results for both methods were within established NCCLS ranges.
4. Mean colony counts overall ranged from 2.60-7.91 × 10^5 CFU/mL). The largest variation between methods was not extremely different and occurred with *S epidermidis* (mean NCCLS colony count = 2.60 × 10^5 and SRGA = 6.75 × 10^5). However, the effect of higher inoculum concentrations would be higher MICs, not lower MICs, as is what is seen with *S epidermidis* SRGA MICs compared to NCCLS MICs.

Table 1. Percentage Doubling Dilution Difference of Daptomycin SRGA MIC Results Compared With NCCLS MIC Results

	Doubling Dilution Difference Compared to NCCLS: –5	–4	–3	–2	–1	0	1	2	3	4	5	% Essential Agreement
All organisms n=242*		1	1	8	92	116	24					95.5
E faecium n=30				1	12	13	4					96.7
E faecalis n=28			1	1	19	5	2					92.9
S aureus n=50				1	11	33	5					98.0
S epidermidis n=48				5	33	10						89.6
Viridans strep. n=36					2	23	11					100
S agalactiae n=25					5	20						100
S pyogenes n=25		1			10	11	3					96.0

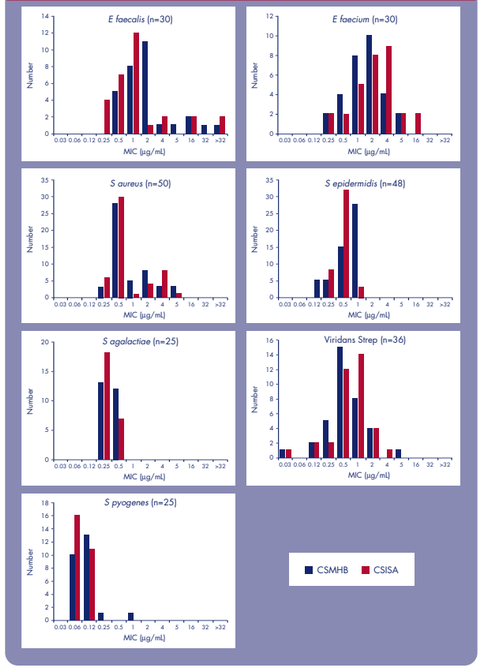
*Organisms with MICs ≤ or > were not included in this analysis

Table 2. Percentage Doubling Dilution Difference of Vancomycin SRGA MIC Results Compared With NCCLS MIC Results

	Doubling Dilution Difference Compared to NCCLS: –5	–4	–3	–2	–1	0	1	2	3	4	5	% Essential Agreement
All organisms n=234*			1		56	167	10					99.6
E faecium n=22					1	18	3					100
E faecalis n=28					16	12						100
S aureus n=50					12	35	3					100
S epidermidis n=48					17	31						100
Viridans strep n=36					2	30	4					100
S agalactiae n=25						25						100
S pyogenes n=25			1		8	16						96.0

*Organisms with MICs ≤ or > were not included in this analysis

Figure 1. Frequency Distribution of Daptomycin MICs Using Calcium Supplemented Mueller Hinton Broth (CSMHB) and Calcium Supplemented IsoSensitest Agar (CSISA)



Conclusions

- Because of the number of variables that influence MIC testing, a +/– 1 doubling dilution is the expected range of results when performing replicate testing of the exact same method.
- Slightly over 95% of NCCLS and SRGA results were within +/– 1 doubling dilution and indicate very good correlation of the 2 methods.
- SRGA *E faecalis* and *S epidermidis* MICs are often more than 1 dilution lower than NCCLS, and therefore, essential agreement rates for these 2 species were somewhat lower.
- *S epidermidis* was the only species where a more systematic difference could be detected. There was no evidence of a systematic difference with vancomycin.
- SRGA results in this study indicate that IsoSensitest Agar supplemented with calcium to levels of 50 µg/mL is a reliable medium for daptomycin MIC testing.