

## Comparison of daptomycin and vancomycin MIC results by NCCLS, SFM, DIN and SRGA methods

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### ABSTRACT

**Objectives:** We determined if daptomycin and vancomycin MIC results obtained with DIN (Deutsches Institut für Normung), SFM (Société Française de Microbiologie), and SRGA (Swedish Reference Group for Antibiotics) are similar to NCCLS (National Committee for Clinical Laboratory Standards) for selected Gram-positive organisms. **Methods:** Approximately 250 enterococci, staphylococci, and streptococci organisms were tested at three different European laboratories. Both clinical and common stock isolates were tested using media supplemented to 50 mg/L calcium. Test specific media were as follows: NCCLS – Mueller Hinton Broth, SRGA – PDM agar, DIN – Isosensitest broth and SFM – Mueller Hinton agar. Quality control strains *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) were tested on each day. **Results:** Essential Agreement Rates (EA% ±1 doubling dilution) compared to NCCLS MICs were:

MIC Method	n	Daptomycin EA (%)	n	Vancomycin EA (%)
DIN	266	99.6	260	100
SFM	259	99.2	253	99.6
SRGA	244	93.0	239	99.6

DIN and SFM daptomycin MICs were within ±1 dilution of NCCLS MICs for all species except *S. pyogenes* (DIN EA% = 96.3, SFM EA% = 93.1). SRGA daptomycin MICs were within ±1 dilution for 95% of strains except *E. faecalis* (EA% = 82.1) and *E. faecium* (EA% = 75.9).

**Conclusions:** There was excellent correlation of daptomycin and vancomycin NCCLS MICs compared to DIN and SFM MICs. There was good correlation of NCCLS and SRGA MICs, with the exception of enterococcal MICs, which will require further study.

### INTRODUCTION

Daptomycin is a cyclic lipopeptide, a new class of antimicrobial agent which depolarizes the bacterial cytoplasmic membrane resulting in release of intracellular potassium ions and cell death. Daptomycin exerts potent bactericidal activity against Gram-positive pathogens, including multi-resistant isolates. Maximum *in vitro* activity is achieved in the presence of a physiological level of calcium ions (Ca<sup>2+</sup>). Standardisation of antimicrobial susceptibility testing is important as it enables comparisons between laboratories and surveillance studies of resistance trends. Although the National Committee for Clinical Laboratory Standards (NCCLS) guidelines<sup>1</sup> prevail in the United States, several different susceptibility testing methods are used in Europe including Deutsches Institut für Normung (DIN)<sup>2</sup>, Société Française de Microbiologie (SFM)<sup>3</sup> and Swedish Reference Group for Antibiotics (SRGA).<sup>4</sup> The objective of the present study was to determine if the minimum inhibitory concentrations (MICs) of daptomycin and vancomycin obtained with DIN, SFM and SRGA methodologies are similar to results obtained using NCCLS guidelines for representative isolates of *Enterococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp. All media were supplemented to give a final concentration of 50 mg/L for calcium ions.

### MATERIALS AND METHODS

**Isolates**  
A total of approximately 250 clinical and stock Gram-positive organisms, comprising *Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, viridans group streptococci, *Streptococcus agalactiae* and *Streptococcus pyogenes* were tested at three laboratories in Germany, France and Sweden. One half of the strains were stock isolates which were tested at each of the three laboratories and the other half of the strains were clinical isolates unique to each site. The number of organisms tested at each laboratory are detailed in Tables 2 - 7.

**MIC testing procedures**  
MICs of daptomycin and vancomycin were determined using four methods (NCCLS, DIN, SFM and SRGA) as detailed in Table 1. *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) were tested on each day as quality control strains. Each laboratory tested all organisms by their country method and by the NCCLS method. Test concentrations of daptomycin and vancomycin were adjusted according to method and bacterial species as follows:

Antimicrobial	Methodology	MIC test range (mg/L)		
		Staphylococci	Enterococci	Streptococci
Daptomycin	NCCLS	0.008 – 16	0.008 – 16	0.008 – 16
	DIN	0.008 – 16	0.008 – 16	0.008 – 16
	SFM	0.008 – 8	0.03 – 32	0.03 – 4
	SRGA	0.03 – 16	0.03 – 32	0.03 – 4
Vancomycin	NCCLS	0.06 – 128	0.06 – 128	0.06 – 128
	DIN	0.06 – 128	0.06 – 128	0.06 – 128
	SFM	0.06 – 16	0.12 – 128	0.06 – 4
	SRGA	0.06 – 16	0.12 – 128	0.06 – 4

**Data analysis**  
MICs as determined using DIN, SFM and SRGA methods were compared with MICs as determined using the NCCLS method. Essential agreement rates (percent of MIC results within ±1 doubling dilution of the NCCLS standard) were calculated for the DIN, SFM SRGA methods. Geometric mean MICs were calculated for both daptomycin and vancomycin against each bacterial species.

Table 1. Summary of MIC determination procedures

Laboratory	Method	Media*	Inocula and Incubation
All three laboratories	NCCLS	Calcium Adjusted Mueller Hinton Broth and Sensititre dried plates (additional 25 mg/L calcium added in wells) (Trek, East Grinstead, UK)	5 x 10 <sup>5</sup> CFU/mL. 24 hours at 35°C in an ambient incubator.
Institute für Med. Mikrobiologie und Infektions Epidemiologie, Leipzig, Germany	DIN	Isosensitest Broth (Oxoid, Basingstoke, UK) supplemented to 25 mg/L calcium and Sensititre dried plates (additional 25 mg/L calcium added in wells) (Trek, East Grinstead, UK)	5 x 10 <sup>5</sup> CFU/mL. 24 hours at 35°C in an ambient incubator.
University Hospital Cote de Nacre, Caen, France.	SFM	Mueller Hinton Agar (bio Merieux, Marcy l'Etoile, France) supplemented to 50 mg/L calcium.	10 <sup>4</sup> CFU/spot. 18 - 24 hours at 37°C in an ambient incubator (pneumococci incubated in 5% CO <sub>2</sub> ).
Swedish Institute for Infectious Disease Control, Solna, Sweden.	SRGA	PDM Agar (AB Biodisk, Solna, Sweden) supplemented to 50 mg/L calcium.	5 x 10 <sup>5</sup> CFU/spot. 20 hours at 36°C in an ambient incubator (pneumococci incubated in 5% CO <sub>2</sub> ).

\* For *S. pneumoniae* testing, all media were supplemented with 5% horse blood

Figure 1. Cumulative percentage of daptomycin and vancomycin DIN and NCCLS results at each MIC (All study isolates, n=266)

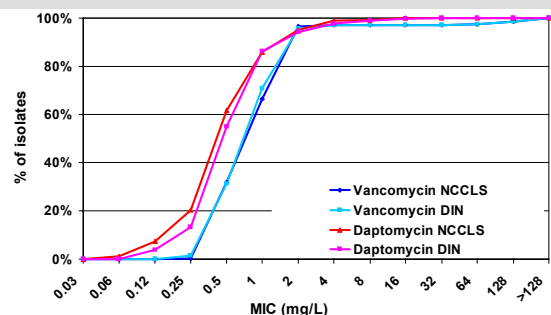


Figure 2. Cumulative percentage of daptomycin and vancomycin SFM and NCCLS results at each MIC (All study isolates, n=261)

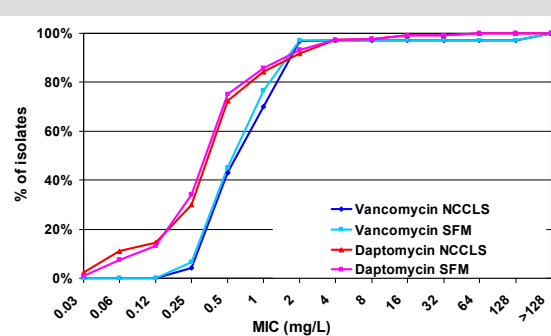
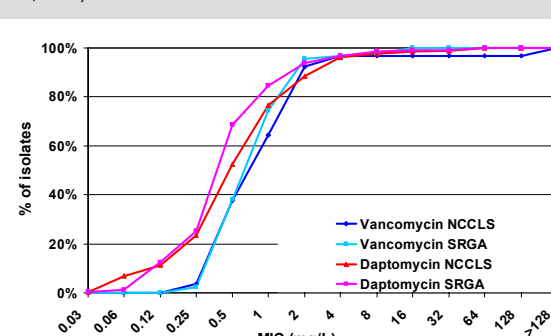


Figure 3. Cumulative percentage of daptomycin and vancomycin SRGA and NCCLS results at each MIC (All study isolates, n=247)



### RESULTS

#### MIC distributions of daptomycin and vancomycin

- The cumulative MIC distributions of daptomycin and vancomycin as determined by DIN, SFM and SRGA methodologies relative to NCCLS methodology against all isolates of enterococci, staphylococci and streptococci are shown in Figures 1-3 respectively.
- Overall, and irrespective of test method, daptomycin was approximately two-fold more active than vancomycin.
- Examination of the graphs indicated good correlation between NCCLS and DIN methodologies (Figure 1) and between NCCLS and SFM methodologies (Figure 2) for both daptomycin and vancomycin. Correlations appeared poorer between NCCLS and SRGA (Figure 3).

#### Comparison of DIN, SFM and SRGA results with NCCLS results

- DIN.** The DIN method produced results that gave the highest agreement with NCCLS results; the essential agreement rates for daptomycin and vancomycin were ≥96.3% (Table 2) and 100% (Table 3) respectively, for all Gram-positive genera tested.
- SFM.** The SFM method produced results that gave high agreement with NCCLS results; the essential agreement rates for daptomycin and vancomycin were ≥93.1% (Table 4) and 98.0% (Table 5) respectively, for all Gram-positive genera tested.
- SRGA.** The SRGA method produced daptomycin results that gave the poorest agreement with NCCLS results (Table 6); the essential agreement rates falling to 75.9% - 96% for five of the seven taxa tested. Results for vancomycin were higher with essential agreement rates of ≥98.0% for all Gram-positive genera tested (Table 7).

#### Comparative activity (MIC mg/L) of daptomycin and vancomycin

- Geometric mean MICs, as determined using NCCLS methodology, for daptomycin and vancomycin against each bacterial species are shown in Table 8.
- Both agents were highly active (geometric mean MICs <2 mg/L) against all the staphylococci and streptococci.
- Daptomycin was more active than vancomycin against *E. faecalis*, *S. aureus*, *S. epidermidis*, *S. agalactiae* and *S. pyogenes* according to NCCLS results from all of the laboratories. Daptomycin and vancomycin had similar activity against Viridans group streptococci.

Table 2. Percentage doubling dilution difference of daptomycin DIN MIC results compared to NCCLS MIC results

	No. of isolates	% Doubling Dilution Difference Compared to NCCLS					Essential Agreement	
		-3	-2	-1	0	1		
All Strains	266			8.3	62.4	28.9	0.4	99.6%
<i>E. faecium</i>	31			12.9	61.3	25.8		100%
<i>E. faecalis</i>	32			9.4	81.3	9.4		100%
<i>S. aureus</i>	55			9.1	65.5	25.5		100%
<i>S. epidermidis</i>	54			5.6	66.7	27.8		100%
Viridans streptococcus	41			17.1	61.0	22.0		100%
<i>S. agalactiae</i>	26				46.2	53.8		100%
<i>S. pyogenes</i>	27				44.4	51.9	3.7	96.3%

Table 4. Percentage doubling dilution difference of daptomycin SFM MIC results compared to NCCLS MIC results

	No. of isolates	% Doubling Dilution Difference Compared to NCCLS					Essential Agreement	
		-3	-2	-1	0	1		
All Strains	259			19.3	65.6	14.3	0.4	99.2%
<i>E. faecium</i>	28			32.4	64.3	3.6		100%
<i>E. faecalis</i>	31			12.9	80.6	6.5		100%
<i>S. aureus</i>	51			33.3	64.7	2.0		100%
<i>S. epidermidis</i>	51			29.4	66.7	3.9		100%
Viridans streptococcus	40			7.5	75.0	17.5		100%
<i>S. agalactiae</i>	29			3.4	55.2	41.4		100%
<i>S. pyogenes</i>	29			3.4	48.3	41.4	3.4	93.1%

\* Strains with daptomycin MICs >16 mg/L were not included in this analysis

Table 6. Percentage doubling dilution difference of daptomycin SRGA MIC results compared to NCCLS MIC results

	No. of isolates	% Doubling Dilution Difference Compared to NCCLS					Essential Agreement			
		-3	-2	-1	0	1				
All Strains	244			1.2	4.9	34.0	41.4	17.6	0.8	93.0%
<i>E. faecium</i>	29			6.9	17.2	51.7	17.2	6.9		75.9%
<i>E. faecalis</i>	28				17.9	57.1	25.0			82.1%
<i>S. aureus</i>	50					28.0	62.0	10.0		100%
<i>S. epidermidis</i>	48			2.1	2.1	62.5	33.3		5.1	95.8%
Viridans streptococcus	39					12.8	56.4	25.6		94.9%
<i>S. agalactiae</i>	25						52.0	48.0		100%
<i>S. pyogenes</i>	25				4	12.0	28.0	56.0		96.0%

\* Strains with daptomycin MICs ≤0.008 mg/L or >16 mg/L were not included in this analysis

Table 8. Summary of geometric mean daptomycin and vancomycin MICs (mg/L) by NCCLS method

	N	Germany			France			Sweden		
		DAP	VAN		DAP	VAN		DAP	VAN	
All strains	266	0.62	1.15 (4)*		0.50 (2)	1.03 (8)		0.71 (3)	1.16 (8)	
<i>E. faecium</i>	31	1.53	2.45 (2)		2.24	2.83 (6)		3.10 (1)	2.83 (6)	
<i>E. faecalis</i>	32	1.48	2.13 (2)		1.67 (2)	1.91 (2)		2.46 (2)	2.52 (2)	
<i>S. aureus</i>	55	0.64	1.16		0.57	1.07		0.69	1.18	
<i>S. epidermidis</i>	54	0.61	1.83		0.47	1.63		0.75	2.03	
Viridans strep	41	0.57	0.59		0.44	0.55		0.56	0.51	
<i>S. agalactiae</i>	26	0.34	0.56		0.26	0.5		0.30	0.5	
<i>S. pyogenes</i>	27	0.15	0.51		0.06	0.39		0.10	0.44	

Numbers in parentheses represent the number of strains with MICs greater than the highest dilution tested. These values were included in the mean calculation as the next highest dilution (e.g. >128 = 256).

DAP = Daptomycin; VAN = Vancomycin

Table 3. Percentage doubling dilution difference of vancomycin DIN MIC results compared to NCCLS MIC results

	No. of isolates	% Doubling Dilution Difference Compared to NCCLS					Essential Agreement	
		-3	-2	-1	0	1		
All Strains	266			11.2	81.2	7.7		100%
<i>E. faecium</i>	31			14.8	85.2			100%
<i>E. faecalis</i>	32			20.0	73.3	6.7		100%
<i>S. aureus</i>	55			14.5	80.0	5.5		100%
<i>S. epidermidis</i>	54			5.6	87.0	7.4		100%
Viridans streptococcus	41			9.8	75.6	14.6		100%
<i>S. agalactiae</i>	26			7.7	73.1	19.2		100%
<i>S. pyogenes</i>	27			7.4	92.6			100%

\* *E. faecalis* with vancomycin MICs >128 mg/L were not included in this analysis

Table 5. Percentage doubling dilution difference of vancomycin SFM MIC results compared to NCCLS MIC results

	No. of isolates	% Doubling Dilution Difference Compared to NCCLS					Essential Agreement	
		-3	-2	-1	0	1		
All Strains	259			18.6	75.1	5.9	0.4	99.6%
<i>E. faecium</i>	28			50.0	37.5	12.5		100%
<i>E. faecalis</i>	31			27.6	65.5	6.9		100%
<i>S. aureus</i>	51			11.8	84.3	3.9		100%
<i>S. epidermidis</i>	51			13.7	80.4	3.9	2.0	98.0%
Viridans streptococcus	40			12.5	87.5			100%
<i>S. agalactiae</i>	29			17.2	82.8			100%
<i>S. pyogenes</i>	29			13.8	65.5	20.7		100%

\* Strains with vancomycin MICs >128 mg/L were not included in this analysis

Table 7. Percentage doubling dilution difference of vancomycin SRGA MIC results compared to NCCLS MIC results

	No. of isolates	% Doubling Dilution Difference Compared to NCCLS					Essential Agreement	
		-3	-2	-1	0	1		
All Strains	239			0.4	18.4	74.9	6.3	99.6%
<i>E. faecium</i>	24				4.2	83.3	12.5	100%
<i>E. faecalis</i>	28				42.9	46.4	10.7	100%
<i>S. aureus</i>	50			2.0	24.0	74.0		98.0%
<i>S. epidermidis</i>	48				33.3	64.6	2.1	100%
Viridans streptococcus	39					87.2	12.8	100%
<i>S. agalactiae</i>	25					25.0		100%
<i>S. pyogenes</i>	25				12.0	76.0	12.0	100%

\* Strains with vancomycin MICs ≤0.06 mg/L or >128 mg/L were not included in this analysis

### CONCLUSIONS

- Daptomycin is more active than vancomycin against *E. faecalis*, *S. aureus*, *S. epidermidis*, *S. agalactiae* and *S. pyogenes*.
- There is excellent correlation between MIC results obtained by NCCLS and DIN methodologies and between NCCLS and SFM methodologies, for both daptomycin and vancomycin.
- There is good correlation between daptomycin MIC results obtained by NCCLS and SRGA methodologies for *Staphylococcus* spp. and *Streptococcus* spp. but poorer correlation for *E. faecium* and *E. faecalis*.

### REFERENCES

- National Committee for Clinical Laboratory Standards. Approved