C-315

Reproducibility of Daptomycin MIC Results Using Dry-Form Commercial Trays with Supplemental Calcium Content

RN JONES, PR RHOMBERG, HS SADER, TR FRITSCHE JMI Laboratories, North Liberty, IA, USA

ASM 2005

JMI Laboratories

North Liberty, IA, USA

www.jmilabs.com

319.665.3370, fax 319.665.3371

ronald-jones@jmilabs.com

ABSTRACT

Background: Daptomycin (DAP) is a cyclic lipopeptide that has demonstrated potent antimicrobial activity against Gram-positive pathogens including oxacillin-resistant (R) staphylococci, penicillin-R streptococci and vancomycin-R enterococci. Accurate broth microdilution (BMD) susceptibility testing requires supplemental concentrations of calcium (Ca) in the medium (50 mg/L) and concurrent quality control. We evaluated the reproducibility of DAP MIC results tested in dry-form commercial BMD panels (TREK Diagnostics) with long shelf-life and a defined Ca concentration.

Methods: 10 strains including 4 ATCC and 6 clinical strains were susceptibility tested according to NCCLS guidelines for BMD. Each strain was tested 3 times a day for 3 days against DAP and linezolid (LZD, control). Target reproducibility was defined as $\geq 95\%$ of MIC results within $\pm 1 \log_2$ dilution for same day and between day results as defined by the NCCLS M23-A2 and the FDA.

Results: Reproducibility tests for DAP produced 87.8 and 84.4% identical results for same day testing and between day replicates, respectively. For DAP, 100% of MIC values were within ± 1 log₂ dilution of the established MIC mode for each strain. On-scale MIC values were observed for DAP and LZD for all test strains except for DAP MIC values against 2 *S. pneumoniae* and 1 *S. pyogenes* isolates. The LZD control agent was also reproducible with 100% of MIC results within ± 1 log₂ dilution step, and 98.9% identical MIC results for both tests. All MIC results for the ATCC strains were within NCCLS published MIC ranges.

Conclusions: The commercial dry-form panels supplemented with calcium 50 mg/L evaluated in the present study provided accurate and reproducible DAP MIC results. These products will allow DAP to be tested in appropriate broth media, while other drugs can be tested in standard cation-adjusted Mueller-Hinton broth medias.

INTRODUCTION

Many recent publications have reported increasing rates of resistance among Gram-positive bacterial species limiting the clinical use of antimicrobial agents including β-lactams versus pneumococci, oxacillin versus staphylococci and vancomycin when testing both staphylococci and enterococci. Daptomycin is a novel cyclic lipopeptide with potent Gram-positive potency resulting in rapid bactericidal activity by cell wall and bacterial membrane targets. No cross-resistance has been demonstrated with any other antimicrobial classes.

Early studies have demonstrated a two- to four-fold increase in daptomycin activity when Mueller-Hinton broth was supplemented with calcium to physiological levels (50 mg/L) when compared to MIC values tested with the CLSI/NCCLS recommended level of 20 - 25 mg/L. Accurate and reproducible daptomycin broth microdilution MIC testing methods are critical to aid clinicians when prescribing daptomycin for the treatment of serious skin and soft tissue infections caused by multidrug-resistant (MDR) Gram-positive pathogens.

To prepare for continued clinical applications of daptomycin, the use of commercially prepared broth microdilution products will be necessary via validation tests that provide accurate and reproducible results and an extended shelf life. We investigated the reproducibility of commercially prepared dry-format broth microdilution MIC panels with supplemental calcium ion at 50 mg/L specifically for daptomycin in vitro testing. This report summarizes the results of reagent reproducibility tests utilizing CLSI/NCCLS M23-A2 guidelines.

MATERIALS AND METHODS

MIC reproducibility testing was assessed utilizing a 10 organism study set including four American Type Culture Collection (ATCC) quality control strains and six clinical isolates (see Table 1). Broth microdilution MIC testing was performed according to Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards [NCCLS]) M7-A6 procedures on commercially prepared dry-form panels (TREK Diagnostics, Cleveland, OH) which were supplemented with additional calcium to achieve the final post inoculation concentration of 50 mg/L for daptomycin susceptibility testing. Linezolid was used as a control, Gram-positive-active agent tested in standard cation-adjusted Mueller-Hinton broth. Streptococci were tested in cation-adjusted Mueller-Hinton broth supplemented with 2 - 5% lysed horse blood. Each strain was tested three times daily for three days to generate 90 results allowing within and between day reproducibility analysis. Acceptable target reproducibility was defined as $\geq 95\%$ of MIC results within \pm one log_2 dilution of the modal MIC for both same day and between day results, and greater than 50% of MIC results should be identical to the mode.

RESULTS

- Table 1 summarizes the daptomycin MIC reproducibility results tested in Mueller-Hinton broth supplemented to 50 mg/L calcium ion for the 10 strains with 87.8 and 84.4% identical results for same day and between day tests, respectively.
- The linezolid control agent reproducibility MIC results are listed in Table 2 for the 10 strains tested with 98.9% of MIC results identical to the modal value.

- Both daptomycin and linezolid demonstrated excellent reproducibility results with 100.0% of MIC results within ± one log₂ dilution of the modal MIC value.
- All MIC results for daptomycin and linezolid for the four ATCC quality control organisms were within CLSI/NCCLS published ranges.
- On-scale MIC results were observed for all daptomycin and linezolid values except for two *S. pneumoniae* and one *S. pyogenes* isolates for daptomycin MIC results.

Table 2. Results of a broth microdilution MIC reproducibility experiment with linezolid tested against 10 organisms (includes quality control strains) three replicates daily for three consecutive days.

Organism (strain no.)	V	Vithin	daily rep	licates	S ^a	Between days ^b						
	-2	-1	Same	+1	+2	-2	-1	Same	+1	+2		
S. mitis (15-6797A)	0	0	9	0	0	0	0	9	0	0		
S. pyogenes (7-6560A)	0	0	9	0	0	0	0	9	0	0		
S. pneumoniae (13-3602C)	0	0	9	0	0	0	0	9	0	0		
S. pneumoniae (ATCC 49619)	0	0	9	0	0	0	0	9	0	0		
E. faecalis (19-6848A)	0	0	9	0	0	0	0	9	0	0		
E. faecalis (ATCC 29212)	0	0	9	0	0	0	0	9	0	0		
S. epidermidis (1-6777A)	0	0	8	1	0	0	0	8	1	0		
S. aureus (43-507A)	0	0	9	0	0	0	0	9	0	0		
S. aureus (ATCC 29213)	0	0	9	0	0	0	0	9	0	0		
S. aureus (ATCC 25923)	0	0	9	0	0	0	0	9	0	0		
Totals	0	0 °	89°	1°	0	0	0 °	89°	1°	0		

- a. Results compared to the within day consensus MIC (mode or median if three different results were encountered).
- b. Results compared to the all replicate consensus value for each organism (nine results).
- c. Reproducibility was at $100.0\% \pm \text{one } \log_2 \text{dilution step}$, and 98.9% of results were identical (90 total tests).

CONCLUSIONS

- MIC result reproducibility was acceptable for both daptomycin and linezolid with 100.0% of results within ± one log₂ dilution step. Identical daptomycin MIC results were observed for 87.8 and 84.4% of same day and between day tests.
- The commercially prepared dry-format broth microdilution panels with supplemental calcium to 50 mg/L for daptomycin testing demonstrated excellent reproducibility of MIC results according to CLSI/NCCLS testing guidelines.
- Results of these reproducibility tests show commercially prepared dry-format broth microdilution testing can be performed to accurately test daptomycin susceptibility with appropriate broth requirements in clinical sites.

SELECTED REFERENCES

Carpenter CF, Chambers HF. (2004). Daptomycin: Another novel agent for treating infections due to drug-resistant gram-positive pathogens. *Clinical Infectious Disease* 38:994-1000.

Clinical and Laboratory Standards Institute. (2005). *Performance standards for antimicrobial susceptibility testing. Document M100-S-15*. Wayne, PA:CLSI.

Fluit AC, Schmitz FJ, Verhoef J, Milatovic D. (2004). In vitro activity of daptomycin against Gram-positive European clinical isolates with defined resistance determinants. *Antimicrobial Agents and Chemotherapy* 48:1007-1011.

Fuchs PC, Barry AL, Brown SD. (2000). Daptomycin susceptibility tests: Interpretive criteria, quality control and effect of calcium in vitro tests. *Diagnostic Microbiology and Infectious Disease* 38:51-58.

National Committee for Clinical Laboratory Standards. (2001). *Development of in vitro susceptibility testing criteria and quality control parameters. Approved guideline M23-A2*. Wayne, PA:NCCLS.

National Committee for Clinical Laboratory Standards. (2003). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M7-A6*. Wayne, PA:NCCLS.

Streit JM, Jones RN, Sader HS. (2004). Daptomycin activity and spectrum: A worldwide sample of 6,737 clinical gram-positive organisms. *Journal of Antimicrobial Chemotherapy* 53:669-674.

Table 1. Results of a broth microdilution MIC reproducibility experiment with daptomycin tested against 10 organisms (includes quality control strains) three replicates daily for three consecutive days.

encountered).

	Variations in log ₂ dilutions for:											
	Within daily replicates ^a						Between days ^b					
Organism (strain no.)	-2	-1	Same	+1	+2		-2	-1	Same	+1	+2	
6. mitis (15-6797A)	0	0	9	0	0		0	0	9	0	0	
S. pyogenes (7-6560A)	0	0	9	0	0		0	0	9	0	0	
S. pneumoniae (13-3602C)	0	0	8	1	0		0	0	8	1	0	
S. pneumoniae (ATCC 49619)	0	0	8	1	0		0	0	5	4	0	
. faecalis (19-6848A)	0	1	8	0	0		0	1	8	0	0	
E. faecalis (ATCC 29212)	0	0	9	0	0		0	0	9	0	0	
6. epidermidis (1-6777A)	0	0	7	2	0		0	0	7	2	0	
6. aureus (43-507A)	0	1	6	2	0		0	1	6	2	0	
S. aureus (ATCC 29213)	0	2	7	0	0		0	2	7	0	0	
S. aureus (ATCC 25923)	0	1	8	0	0		0	1	8	0	0	
otals	0	5°	79°	6°	0		0	5°	76 ^c	9°	0	

. Results compared to the all replicate consensus value for each organism (nine results).

Reproducibility was at 100.0% ± one log, dilution step, and 84.4 - 87.8% of results were identical (90 total