

Evaluation of Dalbavancin MIC Test Strip (MTS) Compared to Broth Microdilution MIC for Relevant Gram Positive Isolates

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Abstract

Background: Dalbavancin is a semi-synthetic lipoglycopeptide antimicrobial agent that was approved by FDA for the treatment of acute bacterial skin and skin structure infections caused by Gram-positive organisms. Similar to other lipoglycopeptide agents, dalbavancin susceptibility testing can be challenging as a result of the large size of the molecule and binding properties. This study was performed to evaluate the performance of a newly developed gradient strip, the dalbavancin MIC Test Strip (MTS, Liofilchem, Roseto degli Abruzzi, Italy) compared to a broth microdilution (BMD) method against indicated Gram-positive isolates. **Methods:** The study isolates tested were strains from clinical sources that included 30 *S. aureus* (including 11 challenge isolates with dalbavancin MICs at or above the susceptible breakpoint of 0.12 mcg/mL), 30 *E. faecalis*, 16 *S. dysgalactiae*, 14 *S. anginosus*, 22 *S. pyogenes*. QC strains (ATCC 29213, ATCC 29212 and ATCC 49619) were tested and results compared to CLSI ranges. Each isolate was tested for dalbavancin MIC by BMD (Sensititre lyophilized plates) and dalbavancin MTS on 100 mm Mueller Hinton agar (MHA) plates for *S. aureus* and *E. faecalis* and MHA+5% sheep blood for streptococci. For a subset of 11 challenge *S. aureus*, results were also compared to modal MIC results based on CLSI BMD method with frozen plates. **Results:** As shown in table, dalbavancin MTS and BMD were within +/- one doubling dilution (essential agreement) for all *S. aureus* and *E. faecalis*. For the majority of streptococci, MTS were 1-4 dilutions lower than BMD.

	Dilution difference of Dalbavancin MTS-BMD (n)								Total	EA
	-4	-3	-2	-1	0	1	2	OS		
<i>S. aureus</i>				5	19	6			30	100.0%
<i>E. faecalis</i>				11	14	2		3	30	100.0%
<i>Streptococcus</i>	1	7	28	10	4			3	53	28.0%

OS: Off-scale (not evaluated in essential agreement)
 EA: Essential agreement

Conclusions: Dalbavancin MTS gave 100% essential agreement for *S. aureus* and *E. faecalis*. Dalbavancin MTS for streptococci will need further refinement as results were significantly lower than BMD. Liofilchem dalbavancin MTS appears to be a viable testing device to determine dalbavancin susceptibility for *S. aureus* and *E. faecalis*.

Introduction

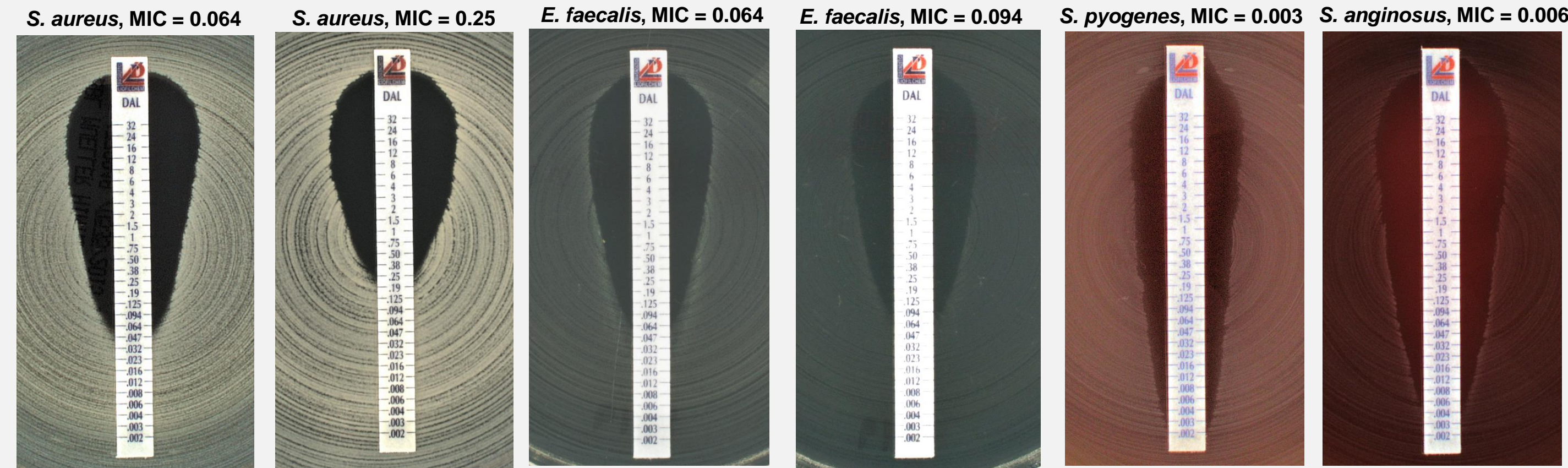
- Dalbavancin, is a semi-synthetic lipoglycopeptide that was approved by the FDA for the treatment of acute bacterial skin and skin structure infections caused by Gram-positive organisms.
- Similar to other lipoglycopeptide agents, dalbavancin susceptibility testing can be challenging as a result of the relatively large size of the molecule and its binding/agar diffusion properties.
- Liofilchem (Roseto degli Abruzzi, Italy) has developed a dalbavancin MIC test strip. The Liofilchem MIC test strip is a quantitative agar-based diffusion assay for determining the minimum inhibitory concentration (MIC) and is available for a variety of different antimicrobial agents.
- This study was performed to compare the dalbavancin MTS MIC to broth microdilution MIC for *Staphylococcus aureus*, *Enterococcus faecalis* and streptococci.

References:

- Clinical and Laboratory Standards Institute. 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 10th ed. Approved standard, CLSI M7-10, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2015. Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard – 25th Edition. CLSI document M100-25 Wayne, PA.
- http://www.liofilchem.net/en/mov_mic_test_strip.php

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Dalbavancin MTS photographs of select study isolates (MIC in µg/mL)



Methods

Study Strains (recent clinical isolates from a variety of sources in US):

- 30 *S. aureus* (including 11 challenge isolates with dalbavancin MICs at or above the susceptible FDA breakpoint of 0.12 mcg/mL and including 4 VISA, 14 MRSA and 12 MSSA)
- 30 *E. faecalis* (including 3 vancomycin resistant)
- 16 *S. dysgalactiae*
- 14 *S. anginosus*
- 22 *S. pyogenes*
- QC strains (*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619)

MIC methods:

Each isolate was tested once at Laboratory Specialists, Inc. by broth microdilution with Sensititre lyophilized panels containing dalbavancin concentrations of 0.004-4 µg/mL (ThermoFisher, E. Grinstead, UK) and by dalbavancin MTS containing concentrations of 0.002-32 µg/mL (Liofilchem, Roseto degli Abruzzi, Italy) on 100 mm Mueller Hinton Agar II plates (Becton Dickinson, Sparks, MD) for *S. aureus* and *E. faecalis* and 100 mm MHA+ 5% sheep blood agar plates (Becton Dickinson, Sparks, MD) for streptococci.

For a subset of 11 *S. aureus*, the dalbavancin BMD and MTS results were compared to 12 replicate CLSI reference BMD results.

Results

- Dalbavancin MTS MIC results for all 3 QC strains were within the CLSI expected ranges. (Table 1)
- For select set of 11 *S. aureus* with target MIC results based on replicate CLSI BMD testing, both Sensititre BMD and MTS results were within +/-1 doubling dilution. (Table 2)
- All dalbavancin MTS MIC results for *S. aureus* were within +/- 1 doubling dilution of the BMD MIC (100% essential agreement). Categorical agreement was 86.7%; with results for 2 isolates considered major errors (BMD MIC of 0.12 µg/mL and MTS MIC of 0.19 µg/mL) and results for 2 isolates considered very major errors (BMD MIC of 0.25 µg/mL and MTS MICs of 0.12 and 0.094 µg/mL). The limited number of isolates and larger % of challenge isolates with MICs near the breakpoint attributed to *S. aureus* categorical agreement <90%. (Figure 1)
- All dalbavancin MTS MIC results for *E. faecalis* were within +/- 1 doubling dilution of the BMD MIC (100% essential agreement). Categorical agreement is not provided as breakpoints are not currently available. (Figure 2)
- Dalbavancin MTS MIC results for streptococci tended to be lower than the BMD MIC, with majority 2 doubling dilutions lower. Categorical agreement was 100%. Validation of detection of resistance was not possible as only susceptible isolates are available. (Figure 3)

Table 1: Dalbavancin MIC results (µg/mL) for QC strains

QC Strain	BMD	MTS	CLSI Expected Range
<i>S. aureus</i> ATCC 29213	0.06	0.06	0.03-0.12
<i>E. faecalis</i> ATCC 29212	0.06	0.047	0.03-0.12
<i>S. pneumoniae</i> ATCC 49619	0.016	0.012	0.008-0.03

Table 2: Dalbavancin Target BMD results compared to study BMD and MTS results for select *S. aureus*

Study Isolate No.	Dalbavancin MIC (µg/mL)		
	Target BMD*	Study BMD	Study MTS
SA-1	0.032	0.064	0.064
SA-2	0.064	0.064	0.064
SA-3	0.5	0.25	0.25
SA-4	0.125	0.25	0.094
SA-5	0.5	0.5	0.25
SA-6	0.5	0.5	0.25
SA-7	0.25	0.25	0.19
SA-8	0.125	0.125	0.19
SA-9	0.5	0.25	0.25
SA-10	0.125	0.125	0.094
SA-11	0.125	0.125	0.125

*Modal MIC of 12 replicates tested by CLSI BMD method

Figure 1. Scatterplot of MTS MIC to BMD MIC for 30 *S. aureus*

MTS MIC (µg/mL)	BMD MIC (µg/mL)					
	0.016	0.032	0.064	0.125	0.25	0.5
0.25					2	2
0.19				2	1	
0.125				1	1	
0.094			1	2	1	
0.064		1	9			
0.047		3		1		
0.032			1			
0.023		1				
0.016	1					

Figure 2. Scatterplot of MTS MIC to BMD MIC for 30 *E. faecalis*

MTS MIC (µg/mL)	BMD MIC (µg/mL)					
	0.032	0.064	0.125	0.25	0.5	>4
>32						3
0.125			1			
0.094		1	2	1		
0.064		7	2			
0.047	1	3	3			
0.032		3				
0.023		3				
0.016						

Figure 3. Scatterplot of MTS MIC to BMD MIC for 53 Streptococci

MTS MIC (µg/mL)	BMD MIC (µg/mL)						
	≤0.004	0.008	0.016	0.032	0.06	0.12	0.25
0.25							
0.12							
0.06							
0.032							
0.016					1		
0.012			1				
0.008		1	2	1			
0.006		2	3	10			
0.004		4	8	5			
0.003	1		8	2			
0.002	2	1		1			

Conclusions

- The dalbavancin MTS against *S. aureus* and *E. faecalis* performed similar to BMD testing.
- All streptococci were correctly categorized as susceptible by dalbavancin MTS MICs, however, most were 1-3 dilutions lower than BMD MICs.
- Additional testing with more isolates, at multiple sites and with multiple MHA is recommended for further validation.
- The dalbavancin MTS strip provided an easy-to-use manual method of dalbavancin MIC determination with very clear endpoints.