Saturday-423

Comparison of Delafloxacin MIC Test Strip and Broth Microdilution MIC Results for *Staphylococcus* spp., *E. faecalis*, *Enterobacteriaceae and P. aeruginosa*

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Abstract (Updated)

Background: Delafloxacin (DLX) is a fluoroquinolone class antimicrobial agent whose NDA is currently under review by the Food and Drug Administration for the oral and IV treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by a variety of bacteria, including MRSA. This study was performed to evaluate the performance of a newly developed gradient strip, the delafloxacin MIC Test Strip (MTS) from Liofilchem, Roseto degli Abruzzi, Italy compared to a broth microdilution method against relevant Gram-negative and Gram-positive isolates. **Methods:** The study isolates (45 *Staphylococcus aureus* [30 MRSA, 15 MSSA], 5 *Staphylococcus epidermidis,* 20 *Enterococcus faecalis,* 24 *Escherichia coli,* 10 *Enterobacter cloacae,* 10 *Klebsiella oxytoca,* 10 *Klebsiella pneumoniae,* 5 *Proteus mirabilis* and 1 *Serratia marcescens*) were recent clinical isolates chosen to include a wide range of DLX MIC results (≤0.004->32 µg/mL). Each isolate was tested for DLX MIC by broth microdilution (BMD; LSI prepared frozen panels) and by DLX MTS on 100 mm Mueller Hinton agar (MHA) plates (Becton Dickinson, Sparks, MD) and a subset of 20 strains was also tested on MHA plates from two additional manufacturers (Hardy, Santa Maria, CA and Remel, Lenexa, KA). QC strains (*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) were tested on each day of testing and results compared to CLSI expected ranges. **Results:** DLX MTS and BMD results (shown in table) were within >90% categorical agreement for all bacterial groups. All quality control results were within CLSI established ranges.

Organism Group	-2	-1	0	1	2	BMD>, both R	MTS>, both R	BMD<, both S	Total	CA
Staphylococcus spp.		3	28	12		3		4	50	92%
E. faecalis		2	15	3					20	100%
Enterobacteriaceae		6	24	19	3	4	4		60	92%
P. aeruginosa			9	5		1	5		20	100%
Streptococcus spp.		13	17	3		1			35	97%

Conclusions: This initial evaluation of the DLX MTS showed good correlation to BMD MIC. Further testing with additional isolates at multiple sites and with media from multiple manufacturers is warranted.

Introduction

- Delafloxacin is a quinolone antimicrobial agent with enhanced antimicrobial activity, including activity against MRSA (methicillin-resistant *Staphylococcus aureus*), a favorable tolerability profile, and administration of both intravenous and oral forms.
- Liofilchem (Roseto degli Abruzzi, Italy) manufactures MIC test strips (MTS) for a variety of antimicrobial agents, including FDA approved vancomycin, dalbavancin, ceftolozane-tazobactam, meropenem, ceftazidime and telavancin. The Liofilchem MIC test strip is a quantitative agar-based diffusion assay for determining the minimum inhibitory concentration (MIC).
- This study was performed as part of a preliminary evaluation of the delafloxacin MTS strip, prior to initiating a U.S. 510(k) study.
- The study compared the delafloxacin MTS MIC to broth microdilution MIC for the anticipated indicated ABSSSI species.

References:

1. 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.
2. Clinical and Laboratory Standards Institute. 2016 Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard – 26th Edition. CLSI document M100-26 Wayne, PA
3. http://www.liofilchem.net/en/mov_mic_test_strip.php

Methods

Study Strains

20 *Enterococcus faecalis* (EF)
45 *Staphylococcus aureus* (30 MRSA, 15 MSSA)
5 *Staphylococcus epidermidis* (SE)
24 *Escherichia coli* (EC)
10 *Enterobacter cloacae* (ECL)
10 *Klebsiella oxytoca* (KO)
10 *Klebsiella pneumoniae* (KP)
5 *Proteus mirabilis* (PM)
1 *Serratia marcescens* (SM)
20 *Pseudomonas aeruginosa* (PA)

35 streptococci :
5 *Streptococcus dysgalactiae* (DYS)
5 *Streptococcus anginosus* (AG)
10 *Streptococcus pyogenes* (PY)
10 *Streptococcus agalactiae* (AG)
5 *Streptococcus mitis/oralis* (MI)

QC strains: *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. pneumoniae* ATCC 49619

Testing site:
Laboratory Specialists, Inc., Westlake, OH

MIC methods:

- All isolates were tested once by BMD according to CLSI method (1) and once by MTS on Becton Dickinson MHA (Sparks, MD) using the same initial suspension (equivalent to 0.5 McFarland standard) for both methods. A subset of 20 non-fastidious isolates (16 Enterobacteriaceae and 4 *P. aeruginosa*) + 4 QC strains and 5 streptococcus + 1 QC strain were tested by MTS on 2 additional commercial media lots (Hardy [Santa Maria, CA] and Remel [Lenexa, KS])
- Quality control strains were tested each day of testing.
- MTS results were rounded up to next doubling dilution for analysis. MIC results were interpreted according to proposed FDA breakpoints.

Results

- Quality Control **(Table 1)**: All delafloxacin BMD and MTS MIC results were within CLSI expected QC ranges.
- Delafloxacin MTS (BD MHA) compared to BMD:

***Staphylococcus* spp. (Figure 1):** MTS were within +/- one dilution of BMD results for 100% of isolates. 8.0% categorical error rate was attributed to minor errors (MTS results for 4 isolates were all higher than BMD)

***E. faecalis* (Figure 2):** MTS were within +/- one dilution of BMD results for 100% of isolates and there were no categorical errors.

***Enterobacteriaceae* (Figure 3):** MTS were within +/- one dilution of BMD results for 88.3% of isolates. 10% of isolates with MTS and BMD results > 1 dilution different were resistant strains with BMD results >32 and MTS 4-16 µg/mL. (See *E. cloacae* picture for example of small ellipse at top concentration) 8.3% categorical error rate was attributed to minor errors.

***P. aeruginosa* (Figure 4):** MTS were within +/- one dilution of BMD results for 75% of isolates. 25% of isolates with MTS and BMD results >1 dilution different were resistant strains with BMD results 4-16 and MTS >32 µg/mL There were no categorical errors.

***Streptococcus* spp.(Figure 5):** MTS were within +/- one dilution of BMD results for 97.1% of isolates. 2.9% categorical error rate was attributed to a major error for one strain with MTS MIC of 0.5 and BMD MIC of 0.25 µg/mL.

- Delafloxacin MTS (Hardy and Remel MHA) subset of 25 isolates compared to MTS (BD MHA) **(Table 2)**:

Hardy MHA: Hardy MHA results were with ±1 dilution of BD MHA results for 88% of isolates. Hardy MHA results were ≥2 dilutions lower compared to BD MHA results for 3 isolates (1 MRSA, 1 *K. oxytoca* and 1 *S. agalactiae*).

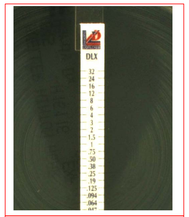
Remel MHA: Remel MHA results were within ±1 dilution of BD MHA results for 92% of isolates. Remel MHA results were ≥2 dilutions lower for 2 MRSA.

Table 1: Quality Control Results

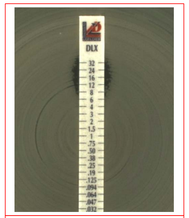
QC Strain	DLX BMD	DLX MTS BD MHA	DFX MTS Hardy MHA	DFX MTS Remel MHA	CFU/mL	CLSI Expected QC Range
E. coli ATCC 29212	0.12	0.06	0.12	0.12	5.65E+05	0.015-0.12
S. aureus ATCC 29213	0.008	0.008	0.004	0.004	5.30E+05	0.001-0.008
S. pneumoniae 49619	0.008	0.016	0.06	0.016	3.65E+05	0.004-0.016
E. coli ATCC 25922*	0.03	0.03	0.03	0.03	6.10E+05	0.008-0.03
P. aeruginosa ATCC 27853	0.25	0.25	0.25	0.25	4.80E+05	0.12-0.5

*Tested 4 times (all MIC results = 0.03 µg/mL); NT - Not Tested

S. aureus
Delafloxacin MIC = 0.19 µg/mL



E. faecalis
Delafloxacin MIC = 0.125 µg/mL



E. cloacae
Delafloxacin MIC = 12 µg/mL

P. aeruginosa
Delafloxacin MIC = 0.19 µg/mL

Figure 1. Delafloxacin MTS MIC compared to BMD MIC for 50 *Staphylococcus* spp.

MTS MIC (µg/mL)	BMD MIC (µg/mL) ≤0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	>4
≤0.002												
0.004	2											
0.008	2	3										
0.015		2	3	1								
0.03				1								
0.06				2	2							
0.125					1	1						
0.25						1	2	2				
0.5							1	2				
1								3	10			
2									1	2		
4										1	2	
8												1
16												
32												
>32												2

Cat. agreement 92.0% Minor error 8.0% Major error 0.0% VM error 0.0%

Figure 2. Delafloxacin MTS MIC compared to BMD MIC for 20 *E. faecalis*

MTS MIC (µg/mL)	BMD MIC (µg/mL) ≤0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	>4
≤0.002												
0.004												
0.008												
0.015												
0.03												
0.06						2	1					
0.125							1					
0.25							1	6	1			
0.5												
1									1			
2										5		
4										2		
8												

Cat. agreement 100% Minor error 0% Major error 0% VM error 0%

Figure 3. Delafloxacin MTS MIC compared to BMD MIC for 60 Enterobacteriaceae

MTS MIC (µg/mL)	BMD MIC (µg/mL) 0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	>16
0.06	1		2								
0.125		6	6	1							
0.25			3	5	1						
0.5			1	1	3	1					
1					1	3					
2						2	4	1			
4							1	2			
8									1		
16								1	4		
32									1		1
>32								1	1	2	3

Cat. agreement 91.7% Minor error 8.3% Major error 0.0% VM error 0.0%

Figure 4. Delafloxacin MTS MIC compared to BMD MIC for 20 *Pseudomonas aeruginosa*

MTS MIC (µg/mL)	BMD MIC (µg/mL) 0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	>16
0.06											
0.125											
0.25			1	3							
0.5					1						
1						3					
2							1				
4								1			
8								2			
16									2		
32											
>32										5	1

Cat. agreement 100% Minor error 0% Major error 0% VM error 0%

Table 2: Comparison of Delafloxacin MTS MIC results on Hardy and Remel MHA compared to BD MHA for 25 strains

MHA	Dilution Difference (compared to BD MHA) >-2	-2	-1	0	1
Hardy	1	2	7	12	3
Remel	1	1	5	16	2
1 MRSA DLX MIC: MTS Hardy=8, MTS Remel=4, MTS BD>32					
1 *K. oxytoca* DLX MIC: MTS Hardy=0.06, MTS BD=0.25					
1 *S. agalactiae* DLX MIC: MTS Hardy=0.008, MTS BD=0.03					
1 MRSA DLX MIC: MTS Remel=0.25, MTS BD=1					

Figure 5. Delafloxacin MTS MIC compared to BMD MIC for 35 *Streptococcus* spp.

MTS MIC (µg/mL)	BMD MIC (µg/mL) ≤0.002	0.004	0.008	0.02	0.03	0.06	0.13	0.25	0.5	1	2	>2
≤0.002												
0.004												
0.008	1	1	5	4								
0.015			2	7	3							
0.03					4	3						
0.06							1					
0.125												
0.25								1				
0.5								1	1			
1												
2											1	
4												

Cat. agreement 97.1% Major error 2.9% VM error 0.0%

Conclusions

- **Overall there was good correlation of delafloxacin MTS MIC results to BMD MIC results.**
- **MTS detection of delafloxacin resistant isolates is excellent for all organism groups, although MTS MIC results for some Enterobacteriaceae and *P. aeruginosa* are more than 1 dilution lower than BMD MIC results.**
- **Since the completion of this study, a multi-lab 510(k) study have been performed; the data is under review and pending submission.**