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Comparison of Lefamulin MIC Test Strip (MTS) to Broth Microdilution MIC for *Streptococcus* spp., *S. aureus*, *M. catarrhalis*, and *Haemophilus* spp.

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Background

Lefamulin is a novel oral and intravenous (IV) pleuromutilin approved in Europe and the U.S. for community-acquired bacterial pneumonia (CAP) in adults with *S. aureus, S. pneumoniae* and *H. influenzae*. Lefamulin MIC Test Strip (MTS; Liofilchem) is a gradient susceptibility testing method that is FDA cleared and CE marketed. The objective of this study was to compare MTS to reference broth microdilution (BMD) for lefamulin susceptibility testing.

Methods

- Three sites tested clinical isolates (N=757) and one site tested challenge isolates (N=142) (see Table 1 for species and isolate numbers tested)
- MIC testing was performed by reference BMD and MTS (Liofilchem, Italy) using cation adjusted Mueller Hinton broth (CAMHB), and CLSI and EUCAST media for the fastidious organisms as shown below
- Each site tested 10 *S. aureus*, 5 *S. pneumoniae* and 5 *H. influenzae* clinical isolates by MTS in triplicate for 3 days for reproducibility and 20 MTS and BMD replicates for 3 QC strains (*S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247)

	CLSI media		EUCAST media	
	BMD-broth	MTS-agar	BMD-broth	MTS-agar
Haemophilus spp.	HTM	HTM	MH-F	MH-F
Streptococcus spp.	CAMHB+5% LHB	MHB+5% SB	MH-F	MH-F

HTM-Haemophilus Test Media, MH-F broth-CAMHB+5% LHB+20 mg/L ß-NAD, MH-F agar-MHA+5% defibrinated Horse Blood+20 mg/L NAD, LHB-lysed horse blood, SB-sheep blood
Source: CLSI M7-ED11E, CLSI100-Ed32E; EUCAST: Media preparation

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Results

- QC: All results were within expected ranges for all 3 QC strains
- Reproducibility: 100% of *S. aureus and H. influenzae*, and 98.5% (CLSI) and 99.3% (EUCAST) of *S. pneumoniae* within ±1 2-fold dilution of modal MIC
- Results for all clinical and challenge strains by species are shown in Table 1
- Figures 1 and 2 show BMD compared to MTS for *S. aureus* and *S. pneumoniae*
- 435 *S. aureus:* EA (essential agreement) = 98.2%; CA (categorical agreement) = 100%
- 183 *S. pneumoniae:* CLSI EA = 97.8% and CA = 100%; EUCAST EA% = 98.4 and CA = 99.4%

Table 1: Lefamulin MIC summary of MTS compared to BMD by organism species and media

Organism species	CLSI BMD (mg/L)	MTS (CLSI MHA) VS. CLSI BMD		MTS (EUCAST MH-F) VS. CLSI BMD		MTS (EUCAST MH-F) VS. EUCAST BMD	
	$MIC_{50/90}$	n	%EA	n	%EA	n	%EA
S. aureus	0.12/0.12	435	98.2	NA	NA	NA	NA
M. catarrhalis	0.12/0.25	28	100	NA	NA	NA	NA
H. influenzae	1/2	116	99.1	116	98.3	116	98.3
H. parainfluenzae	2/4	28	100	28	78.6	28	82.1
S. pneumoniae	0.25/0.25	183	97.8	180	98.4	178	98.9
S. agalactiae	0.03/0.06	20	75.0	20	75	17	76.5
S. anginosus	0.25/0.5	26	100	26	100	23	100
S. mitis	0.12/0.5	15	93.3	15	100	14	100
S. pyogenes	≤0.016/0.03	33	100	33	93.9	24	95.8
S. salivarius	0.12/0.12	15	100	15	100	13	84.6

NA - not applicable; EA - essential agreement (within ± 1 2-fold dilution of reference MIC)

Figure 1: BMD MIC compared to MTS MIC (mg/L) for 435 *S. aureus*

MTS Results	BMD Reference Results														
	≤0.016	0.03	0.06	0.12	0.25 S	0.5 NS	1	2	4	8	16	32	64	128	≥256
≤0.016															
0.03		12	10	3											
0.06		1	129	181	2										
0.12			3	55	17										
0.25 S				1	1										
0.5 NS															
1							1	1	1						
2								1	5	1					
4									1	1					
8											2				
16												1			
32													2	1	
64														1	
128															
≥256															1

The red dotted line represents the CLSI and EUCAST susceptible breakpoint

Figure 2: BMD MIC compared to MTS MIC (mg/L) for CLSI and EUCAST recommended media

(a) *S. pneumoniae* CLSI media n=183

MTS Results (CLSI)	BMD Reference Results (CLSI)														
	≤0.016	0.03	0.06	0.12	0.25	0.5 S	1 NS	2	4	8	16	32	64	128	≥256
≤0.016		2													
0.03	2														
0.06		3	6	17	3										
0.12		1	2	35	22										
0.25				21	46	3									
0.5 S					11	7									
1 NS							1								
2							1								
4															
8															
16															
32															
64															
128															
≥256															

(b) *S. pneumoniae* EUCAST media n= 178

MTS Results (MHF)	BMD Reference Results (MHF)														
	≤0.016	0.03	0.06	0.12	0.25	0.5 S	1 NS	2	4	8	16	32	64	128	≥256
≤0.016	1	1													
0.03	2		1												
0.06			7	11											
0.12		1	3	37	19										
0.25			1	17	53	4									
0.5 S					8	9									
1 NS						1	1								
2							1								
4															
8															
16															
32															
64															
128															
≥256															

The red dotted line represents the CLSI and EUCAST susceptible breakpoint

Results

- Figure 3 shows BMD and MTS MIC results for *H. influenzae* and *Streptococcus* spp. (except *S. pneumoniae*)
- *H. influenzae*: CLSI EA = 99.1% and CA = 98.3% and EUCAST EA = 98.3%
- *Streptococcus* species (except *S. pneumoniae*): CLSI EA = 92.5% and EUCAST EA = 93.7%

Figure 3: BMD Reference MIC compared to MTS MIC (mg/L) for CLSI and EUCAST recommended media

(a) *H. influenzae* CLSI media n=116

MTS Results (CLSI)	BMD Reference Results (CLSI)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2 S	4 NS	8	16	32	64	128	≥256
≤0.016	1														
0.03															
0.06			1												
0.12				1											
0.25					2	4									
0.5						29	10								
1					1	10	32	6							
2 S							2	11							
4 NS								2	3						
8															
16										1					
32															
64															
128															
≥256															

Red dotted line represents CLSI susceptible breakpoint

(b) *H. influenzae* EUCAST media n=116

Test Results (MHF)	Reference Results (MHF)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
≤0.016	1														
0.03															
0.06			1												
0.12			1												
0.25					1	6									
0.5						25	8	1							
1						8	26	10							
2							6	12							
4								3	5						
8								1							
16										1					
32															
64															
128															
≥256															

(c) *Streptococcus* spp. (except *S. pneumoniae*) CLSI media n=109

MTS Results (CLSI)	BMD Reference Results (CLSI)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
≤0.016	10														
0.03	19	15													
0.06		6	7	7											
0.12			8	10	2										
0.25				6	3										
0.5				1	2	4									
1						2	1								
2								1							
4															
8															
16															
32															
64												1			
128															
≥256												1	3		

(d) *Streptococcus* spp. (except *S. pneumoniae*) EUCAST media n= 91

MTS Results (MHF)	BMD Reference Results (MHF)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
≤0.016	10														
0.03	15	7													
0.06	1	4	7	5											
0.12	2		6	5	2										
0.25				10	3										
0.5					1	2									
1						4	2								
2															
4															
8															
16															
32												1			
64													1		
128													1		
≥256													2		

Conclusion

Overall, there is good correlation of MIC results generated with MTS and BMD for lefamulin against the common CAP pathogens *S. aureus*, *S. pneumoniae* and *H. influenzae* (>97% EA and CA)