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Multi-Site Evaluation of Meropenem/Vaborbactam MIC Test Strip (MTS) Compared To Broth Microdilution MICs

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Abstract:

Background: MIC Test Strips (MTS, Liofilchem, Roseto degli Abruzzi, Italy) consists of specialized paper impregnated with a pre-defined concentration gradient of an antimicrobial agent, which is used to determine the minimum inhibitory concentration against bacteria as tested on agar media using overnight incubation and manual reading procedures. Meropenem/vaborbactam (M/V) was recently approved in the US for the treatment of patients 18 years of age and older with complicated urinary tract infections (cUTI) including pyelonephritis caused by the following susceptible microorganisms: *Escherichia coli, Klebsiella pneumoniae,* and *Enterobacter cloacae* species complex. This study was performed to evaluate the performance of M/V MTS compared to a broth microdilution method (BMD) for FDA 510(k) submission. **Methods:** Clinical and challenge isolates were tested by M/V BMD with frozen panels (according to CLSI M7-A10 and M100-S27) and by M/V MTS. Clinical isolates were collected and tested at 3 sites, 10 reproducibility isolates/agent were shared and tested in triplicate on 3 days at 3 sites and challenge isolates were tested at 1 site. Challenge isolates included majority with MIC results near or above the susceptible breakpoint. The total number of clinical isolates tested were: 390 Enterobacteriaceae and 75 *Pseudomonas aeruginosa*. The total number of challenge isolates tested were: 88 Enterobacteriaceae and 25 *P. aeruginosa*. QC strains (*K. pneumoniae* ATCC BAA-1705 and *P. aeruginosa* ATCC 27853) were tested a minimum of 20 times by each site. **Results:** As shown in the table, M/V MTS MIC results for consolidated clinical and challenge organisms were within +/- one doubling dilution (essential agreement) of BMD MIC results for >90% of isolates. For reproducibility strains, 97% of M/VMTS results were within a doubling dilution of BMD results. All MTS and BMD QC results were within CLSI ranges.

Organism	N	% Essential Agreement	% Category Agreement
Enterobacteriaceae	478	95.6	97.1
P. aeruginosa	100	96.0	NA

Conclusions: The M/V MTS against clinically indicated Enterobacteriaceae species and *P. aeruginosa* perform similar to the reference broth microdilution method. The M/V MTS received clearance by FDA, Center for Devices and Radiological Health, for testing of relevant Enterobacteriaceae species.

Introduction

- Liofilchem (Roseto degli Abruzzi, Italy) manufactures MIC test strips (MTS) for a variety of antimicrobial agents, including meropenem/vaborbactam. 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 510(k) study (for "in vitro diagnostic use" label in the U.S.)
- This study compared the meropenem/vaborbactam (M/V) MTS MIC to broth microdilution MIC for the indicated Gram negative organisms (*Enterobacter cloacae* species complex, *Escherichia coli and Klebsiella pneumoniae)* and for additional Enterobacteriaceae species included in "in vitro only list" of the M/V label *(Citrobacter freundii, Citrobacter koseri, Enterobacter aerogenes, Klebsiella oxytoca, Proteus mirabilis, Providencia* spp.*, Serratia marcescens)*

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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. 2017 Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard – 27th Edition. CLSI document M100-276 Wayne, PA
3. http://www.liofilchem.net/en/mov_mic_test_strip.php

Methods

Study Strains (Clinical isolates collected at three sites and challenge isolates)

Organism Group	Clinical	Challenge
C. freundii	15	
C. koseri	12	
E. aerogenes	15	
E. cloacae	90	15
E. coli	120	30
K. oxytoca	12	
K. pneumoniae	90	31
Proteus mirabilis	10	11
P. rettgeri	9	
S. marcescens	12	1
Enterobacteriaceae	390	88
P. aeruginosa	75	25
TOTAL	465	113

All clinical isolates were collected within one year of testing and at least 50% were tested within 7 days of collection.

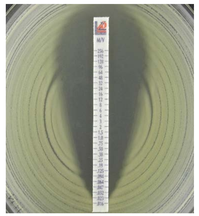
QC strains:
P. aeruginosa ATCC 27853
K. pneumoniae ATCC BAA-1705

The number of challenge organisms with molecular characterized resistance mechanisms were:
8 CTX-M, 7 AmpC, 10 NDM, 8 VIM, 4 KPC, 2 OXA-48, 1 OXA-1, OXA-30

Reproducibility isolates:
10 isolates (4 *E. coli*, 3 *K. pneumoniae*, 2 *E. cloacae* and 1 *P. aeruginosa*) were tested by M/V MTS at each of the 3 testing sites in triplicate on three separate days for a total of 27 results/reproducibility isolate

Testing sites:
- Laboratory Specialists, Inc. (LSI), Westlake, OH
- University of Rochester Medical Center, Rochester, NY
- Cincinnati Children's Hospital & Medical Center, Cincinnati, OH

MIC methods:
- Each isolate was tested once by broth microdilution according to CLSI method (1) with frozen panels containing meropenem/vaborbactam concentrations of 0.016/8-256/8 µg/mL and by meropenem/vaborbactam (M/V (3) containing concentrations of 0.016/8-256/8 µg/mL (Liofilchem, Roseto degli Abruzzi, Italy) on 100 mm Mueller Hinton Agar II plates (MHA from Becton Dickinson [Sparks, MD]. Challenge isolate testing was performed by one site (LSI)
- Quality control strains were tested each day of testing and a total 20 replicates/site were tested.
- MTS results were rounded up to next doubling dilution for analysis. MIC results were interpreted according to FDA breakpoints.



E. coli
Meropenem/vaborbactam
MIC = 0.023/8 µg/mL,
reported as 0.03/8 µg/mL

Results

- Quality Control (Table 1): All M/V MTS MIC results for both QC strains were within the CLSI expected ranges. The majority (95.1%) of M/V MTS MIC results for *K. pneumoniae* ATCC BAA-1705 were 0.06 µg/mL (at the high end of the expected range).
- Reproducibility (Figure 1): 97% of consolidated isolate and site M/V MTS MICs were within +/-1 doubling dilution of modal MIC.
- Clinical & Challenge MTS (Figures 2-5): MTS MIC results were within +/- 1 doubling dilution for 457/478 Enterobacteriaceae, which included 139/150 *E. coli,* 104/105 *E. cloacae* species complex and 115/121 *K. pneumoniae*. Category agreement for all Enterobacteriaceae was 97.1%, major error rate was 1.1% and minor error rate was 2.5%. MTS MIC results were within +/- one doubling dilution for 96/100 *P. aeruginosa.* Category agreement for *P. aeruginosa* was 89.0%, major error rate was 10% and minor error rate was 1.5%.
- M/V MTS trended up to 1 dilution higher compared to the BMD MICs, particularly at the very low end of the MIC range (i.e. ≤0.016-0.06 µg/mL) and for *P. aeruginosa* across the concentration range.

Table 1. Meropenem-Vaborbactam BMD and MTS Quality Control Results by Testing Site

QC Organism	Expected Result	MIC	Reference BMD Frequency				MTS Frequency			
		µg/mL	Site 1	Site 2	Site 3	All Sites	Site 1	Site 2	Site 3	All Sites
P. aeruginosa ATCC 27853	0.12 - 1	0.06				0				0
		0.12				0				0
		0.25	15	15	15	45			11	11
		0.5	4	4	5	13	19	20	9	48
		1	1	1		2	1			1
		2				0				0
K. pneumoniae ATCC BAA-1705	0.015 - 0.06	0.008				0				0
		0.015				0				0
		0.03	16	20	16	52	1		2	3
		0.06	4	1	4	9	19	21	18	58
		0.12				0				0

Figure 1. Meropenem/vaborbactam MTS MIC results for 10 reproducibility isolates
(number of results based dilution difference compared to test mode)

Reproducibility Strain No., Species	Difference in the number of doubling dilutions between test result and test mode							Test MIC Mode
	Off-Scale	-2	-1	0	1	2	Off-Scale	
	All Sites							
R1, *E. coli*			1	19	7			0.03
R2, *E. coli*				24	3			0.25
R3, *E. coli*				21	6			0.12
R4, *K. pneumoniae*			3	13	11			64
R5, *K. pneumoniae*			1	25	1			4
R6, *E. cloacae*			13	14				1
R7, *P. aeruginosa*				24	3			0.5
R10, *E. coli*				26	1			0.06
R11, *E. cloacae*			1	22	4			0.06
R13, *K. pneumoniae*		8	3	13	3			8
Total	**0**	**8**	**22**	**201**	**39**	**0**	**0**	
Between-site Reproducibility	**262/270 = 97%**							

Figure 2. Meropenem/vaborbactam MTS MIC compared to BMD MIC for 478 Enterobacteriaceae*
(number of results at each MIC)

MTS Results	Reference BMD Results														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
≤0.016	**1**															
0.03	**34**	**107**	**1**													
0.06	**10**	**180**	**35**	**3**												
0.12		**3**	**11**	**14**	**1**	**1**										
0.25				**3**	**1**	**1**										
0.5					**1**	**2**										
1					**1**	**1**	**1**									
2							**1**	**2**	**1**							
4								**2**	**3**	**7**						
8									**3**	**3**	**2**					
16									**1**		**5**	**4**				
32										**1**	**2**	**10**	**3**			
64												**1**	**2**			
128													**1**	**3**	**1**	
256															**1**	
>256												**1**		**3**	**3**	

Overall EA (essential agreement)	457 / 478	95.6%
EA, evaluable results	419 / 430	97.4%
Category agreement	464 / 478	97.1%
Category errors: Minor	13 / 478	2.7%
Category errors: Major	1 / 425	0.2%
Category errors: Very Major	0 / 42	0.0%

*105 *Enterobacter cloacae* species complex, 150 *Escherichia coli, 121 Klebsiella pneumoniae 15 Citrobacter freundii, 12 Citrobacter koseri, 15 Enterobacter aerogenes, 12 Klebsiella oxytoca, 26 Proteus mirabilis, 9 Providencia* spp., *and 13 Serratia marcescens*

Figure 3. Meropenem/vaborbactam MTS MIC compared to BMD MIC for 150 *E. coli* (number of results at each MIC)

MTS Results	Reference BMD Results														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
≤0.016	**1**															
0.03	**20**	**55**														
0.06	**8**	**41**	**6**													
0.12		**1**	**2**													
0.25				**1**												
0.5					**1**											
1				**1**												
2					**1**											
4							**1**	**2**	**1**							
8								**1**								
16																
32																
64																
128																
256																
>256																

Overall EA (essential agreement)	139 / 150	92.7%
EA, evaluable results	118 / 121	97.5%
Category agreement	148 / 150	98.7%
Category errors: Minor	2 / 150	1.3%
Category errors: Major	0 / 142	0.0%
Category errors: Very Major	0 / 7	0.0%

Figure 4. Meropenem/vaborbactam MTS MIC compared to BMD MIC for 121 *K. pneumoniae* (number of results at each MIC)

MTS Results	Reference BMD Results														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
≤0.016																
0.03		**17**														
0.06	**1**	**55**	**13**													
0.12		**1**														
0.25																
0.5					**1**											
1						**1**										
2								**1**								
4								**1**		**1**						
8									**1**	**1**	**1**					
16									**1**		**2**	**3**				
32											**1**	**7**	**2**			
64												**1**	**1**			
128													**1**	**2**	**1**	
256															**1**	
>256												**1**		**2**		

Overall EA (essential agreement)	115 / 121	95.0%
EA, evaluable results	115 / 120	95.8%
Category agreement	117 / 121	96.7%
Category errors: Minor	3 / 121	2.5%
Category errors: Major	1 / 93	1.1%
Category errors: Very Major	0 / 26	0.0%

Figure 5. Meropenem/vaborbactam MTS MIC compared to BMD MIC for 100 *P. aeruginosa* (number of results at each MIC)

MTS Results	Reference BMD Results														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
≤0.016																
0.03																
0.06		**1**	**3**													
0.12			**2**													
0.25			**1**	**11**	**3**											
0.5				**1**	**9**	**10**										
1					**1**	**9**	**1**									
2						**4**										
4							**1**	**2**								
8								**5**	**1**							
16								**1**	**5**	**2**						
32										**3**	**2**					
64										**3**						
128																
256																
>256															**14**	**5**

Overall EA (essential agreement)	96 / 100	96.0%
EA, evaluable results	77 / 81	95.1%
Category agreement	89 / 100	89.0%
Category errors: Minor	10 / 100	10.0%
Category errors: Major	1 / 65	1.5%
Category errors: Very Major	0 / 29	0.0%

Conclusions

- **The meropenem/vaborbactam MTS against Enterobacteriacea performed similar to BMD testing.**
- **The essential agreement for meropenem/vaborbactam MTS against *P. aeruginosa* was well above the 90% acceptance criteria (96%), however, overall there was trend for higher MTS results.**
- **There was also a tendency for one dilution higher meropenem-vaborbactam MIC results compared to BMD MIC results, particularly for Enterobacteriaceae with BMD MIC results of ≤0.016-0.06 µg/mL and with the quality control strains.**
- **The M/V MTS endpoints were easily determined; the testing sites reported no reading issues.**
- **The meropenem/vaborbactam MTS was cleared for in vitro diagnostic use by the FDA.**