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Gepotidacin Liofilchem MIC Test Strip Evaluation against *Enterobacteriaceae*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Neisseria gonorrhoeae*

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Abstract

Background: Gepotidacin (GEP), a first-in-class triazaacenaphthylene antibacterial in clinical development, selectively inhibits bacterial DNA replication via a unique binding mode and has activity against multi drug resistant (MDR) target pathogens. GEP was previously shown to be active in vitro against collections of *E. coli* (EC), *Staphylococcus aureus* (SA), *Staphylococcus saprophyticus* (SS) and *Neisseria gonorrhoeae* (NG) following CLSI methodology. The purpose of this study was to evaluate GEP Liofilchem MIC Test Strips (MTS) against collections of clinical isolates compared with MIC results generated using CLSI M7 reference broth or agar dilution methodology to determine if the Liofilchem MTS strip is an acceptable MIC testing option for future GEP studies.

Methods: GEP MICs were determined against 50 isolates each SA and SS and 122 *Enterobacteriaceae* by broth microdilution with Becton Dickinson (BD) cation-adjusted Mueller Hinton broth (CAMHB) following CLSI guidelines and by MTS methods using BD Mueller Hinton agar (MHA). Quality control (QC) strains were tested on each day of testing as recommended by CLSI. Gepotidacin MIC's were also determined for 99 NG isolates by agar dilution following CLSI guidelines and by MTS using both GC agar supplemented with 1% IsoVitaleX and chocolate agar. A subset of organisms was also tested to assess media from different vendors including Hardy Diagnostics and Remel. Essential agreement (EA) and categorical agreement (CA) rates were determined according to FDA guidance. Preliminary GEP breakpoints for categorical analysis (susceptible/intermediate/resistant) were: Enterobacteriaceae (≤4/8/≥16 µg/mL and ≤8/16/≥32 µg/mL), staphylococci (≤1/-/≥2 µg/mL) and NG (≤1/2/≥4 µg/mL).

Results: QC results were within the established CLSI acceptable ranges in all runs. The overall EA rates ranged from 97%-100% for all of the organisms tested, including NG on chocolate agar. CA ranged from 95%-100% and there were no major or very major errors. NG did not grow on Remel GC media and both EA and CA was 100% for the 23 NG isolates tested using Hardy GC media. All MTS results on Hardy and Remel MHA were within ± one dilution of MTS results on BD MHA for the 20 isolate subsets of staphylococci and *Enterobacteriaceae*.

Conclusions: In accordance with FDA guidelines, an EA and CA of ≥90%, as observed in this evaluation indicates acceptable performance of gepotidacin Liofilchem MTS in comparison to reference methods. The Liofilchem MTS is acceptable for determining gepotidacin MICs in future studies with these organisms.

Introduction

Gepotidacin is a first-in-class triazaacenaphthylene antibacterial in clinical development. Gepotidacin selectively inhibits bacterial DNA replication via a unique binding mode and has activity against most multidrug-resistant (MDR) target pathogens [1]. Pathogens implicated in uncomplicated urinary tract infections include *Enterobacteriaceae* and staphylococci, with the most prevalent pathogen being *E. coli*. *N. gonorrhoeae* (NG) is the pathogen implicated in gonorrhea, the second most prevalent bacterial sexually transmitted infection globally. The purpose of this study was to evaluate utilizing gepotidacin gradient diffusion strips, specifically MIC test strips (MTS) developed by Liofilchem, to assess the *in vitro* activity of gepotidacin against clinical isolates in comparison with results obtained by CLSI M7 reference dilution methodologies [2][3]. This study tested the most recent formulations of Liofilchem gepotidacin MTS strips against clinical *Enterobacteriaceae,* staphylococci and NG isolates and evaluated if the strips were equivalent to MIC results obtained by CLSI M7 reference broth and agar dilution methodologies. Preliminary GEP MIC breakpoints to determine categorical agreement are primarily based on in vitro MIC frequency distributions, animal efficacy data, PK/PD, and human exposure levels of GEP achievable for relevant clinical dosing regimens. These breakpoints will be re-evaluated when clinical data is available.

Methods

Experimental Protocol:

- Reference broth microdilution and agar dilution (AD) was performed according to CLSI guidelines [2][3].
- Liofilchem MTS strips (MTS) were tested according to the manufacturer's instructions (Liofilchem, Italy) using the same inoculum preparation as the reference method.
- Liofilchem strips were also tested on Chocolate agar and compared to reference method for GC.
- Subsets of isolates were tested by reference method and MTS using media from multiple vendors.

Isolates: Selected to provide a range of gepotidacin MIC results and resistance phenotypes:

- 50 *Staphylococcus aureus*, 50 *Staphylococcus saprophyticus*, 122 *Enterobacteriaceae* (102 *E. coli*, 5 *Klebsiella oxytoca*, 5 *Klebsiella pneumoniae*, 5 *Enterobacter cloacae*, 5 *Proteus mirabilis)*, 99 *N. gonorrhoeae*

Quality Control: Followed CLSI guidelines [3] on each day of testing.

- Additional replicates were tested to generate a total of 10 replicates for MTS with: *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *N. gonorrhoeae* ATCC 49226.

Drugs and Materials: Broth microdilution panels were prepared with CAMHB from BD (Sparks, MD) and stored at -70ºC during the course of the study. Gepotidacin MTS testing was performed using prepared Mueller Hinton agar (MHA) plates from BD (Sparks, MD), with a subset of isolates also tested on prepared MHA plates from Remel (Lenexa, KS) and Hardy (Santa Maria, CA). For GC, agar dilution plates and GC agar plates (for MTS testing) were made by LSI and inoculated within 1 hour of production.

Data Analysis: All MTS MIC results were rounded to the next doubling dilution and compared to reference MIC results utilizing dilution difference calculations, scattergram plots and determination of essential and category agreement rates with minor, major and very major error rates according to the Class II controls FDA guidance document [4].

- ≥90% essential agreement and category agreement was necessary for acceptance.

Category agreement was assessed as follows:

Table 1. Preliminary Gepotidacin Breakpoints for categorical assessment (µg/mL)

Organism	Susceptible	Intermediate	Resistant
Enterobacteriaceae (1)	≤4	8	≥16
Enterobacteriaceae (2)	≤8	16	≥32
Staphylococci	≤1	-	≥2
N. gonorrhoeae	≤1	2	≥4

Results

Quality Cotrol:

- All quality control results were within the established CLSI acceptable ranges in all runs.

Figure 1. Gepotidacin MTS (BD MHA) compared to reference BMD for 122 *Enterobacteriaceae* (gepotidacin MIC breakpoints ≤4, 8, ≥16 µg/mL)

MIC Results (MTS) µg/mL	Reference MIC Results (BMD) µg/mL											
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
0.06												
0.12												
0.25		2	1									
0.5				2								
1					1	11	2					
2						2	32	13				
4							4	28	4			
8								1	8	1		
16										3	5	
32											1	
64												1
128												
Total	0	2	1	3	13	38	42	12	4	6	0	1

Figure 2. Gepotidacin MTS (BD MHA) compared to reference BMD for 122 *Enterobacteriaceae* (gepotidacin MIC breakpoints ≤8, 16, ≥32 µg/mL)

MIC (MTS) µg/mL	Reference MIC (BMD) µg/mL											
	0.12	0.25	0.5	1	2	4	8	16	32	64	128
0.12											
0.25	2	1									
0.5			2								
1			1	11	2						
2				2	32	13					
4					4	28	4				
8						1	8	1			
16								3	5		
32									1		
64											1
128											
Total	2	1	3	13	38	42	12	4	6	0	1

Shaded area = isolates with MTS MIC results = to BMD MICs /MTS = MIC test strip; BD = Becton Dickinson; MHA = Mueller Hinton agar; BMD = broth microdilution; EA = essential agreement; CA = category agreement

Figure 3. Gepotidacin MTS (BD MHA) compared to reference BMD for 100 staphylococci (gepotidacin MIC breakpoints ≤1, ≥2 µg/mL)

MIC Results (MTS) µg/mL	Reference MIC Results (BMD) µg/mL												
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
0.06	2	1											
0.12	35	11											
0.25		1	19	2									
0.5			6	19									
1					1								
2													
4						1							
8													
16								1					
32													
64													
128													
≥256												1	
Total	37	13	25	21	1	0	1	0	1	0	0	1	0

Figure 4. Gepotidacin GC agar (BD) MTS MICs compared to reference AD (BD) MICs for 99 *N. gonorrhoeae* (gepotidacin MIC breakpoints ≤1, 2, ≥4 µg/mL)

MTS MICs µg/mL	Reference MIC Results (AD) µg/mL													
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
≤0.016														
0.03		1												
0.06		1	1											
0.12			1	5	3									
0.25				1	27	3								
0.5					14	31	5							
1						2	2							
2														
4														
8														
16														
32														
64													1	
128													1	
Total		2	2	6	44	36	7	0	0	0	0	0	2	0

Table 3. Summary table of gepotidacin MTS (BD MHA) compared to BMD and agar dilution reference MIC results by organism groups

Organism	N	Agreement and Error Rates[1]				
		Essential Agreement %	Category Agreement %	Minor Error %	Major Error %	Very Major Error %
Enterobacteriaceae[2]	122	100	95.1	4.9	0	0
E. coli	102	100	96.1	3.9	0	0
Staphylococcus spp.[3]	100	100	100	0	0	0
S. aureus	50	100	100	0	0	0
N. gonorrhoeae (GC agar)	99	100	100	0	0	0
N. gonorrhoeae (Chocolate agar)	99	97	100	0	0	0

[1]Determined according to Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems (1)
[2]*Enterobacteriaceae* included 102 *E. coli*, 5 *E. cloacae*, 5 *K. oxytoca*, 5 *K. pneumoniae* and 5 *P. mirabilis* (error rates matched for breakpoints in Figures 1 and 2.)
[3]*Staphylococcus* spp. includes 50 *S. aureus* and 50 *S. saprophyticus*
MTS = MIC test strip; BD = Becton Dickinson; MHA = Mueller Hinton agar; BMD = broth microdilution; CAMHB = cation-adjusted Mueller Hinton broth

Table 4. Dilution difference distribution of gepotidacin MTS MICs on MHA from multiple manufacturers compared to gepotidacin broth microdilution (BMD) MICs for a subset of isolates (n=20)

Organism	n	Dilution Difference MTS BD MHA / MTS Hardy MHA /MTS Remel MHA - BMD				
		-2	-1	0	1	2
E. coli	7	1	2, 4, 5	4, 2	1, 1, 1	
E. cloacae	2		2, 1, 2	1		
K. oxytoca	2		2	2, 2		
K. pneumoniae	2		1	2, 2, 1		
P. mirabilis	2	1	1, 2, 1	1		
S. aureus	3		2, 2	3, 1, 1		
S. saprophyticus	2			1, 2, 2	1	

Table 5. Dilution difference of gepotidacin MICs for a subset of isolates (n=23) tested with GC agar from multiple manufacturers

Method Comparison	-1	0	1
BD GC Agar MTS - BD GC Agar AD	2	17	4
BD GC Agar MTS - Hardy GC Agar AD	2	19	2
Hardy GC Agar MTS - BD GC Agar AD	7	14	2
Hardy GC Agar MTS - Hardy GC Agar AD	8	14	1
Hardy GC Agar MTS - BD GC Agar MTS	8	14	1

MTS = MIC test strip; BD = Becton Dickinson; AD =Agar dilution
Note: Remel GC agar was also included in the study however none of the GC isolates grew on these plates. Further evaluation would be required to assess Remel GC agar base.

Conclusions

- Essential and category agreement rates were greater than 90% for all organism groups evaluated.
- There were no major or very major errors reported and minor errors were <5%.
- Chocolate agar may be a viable alternative to CLSI reference GC agar for gepotidacin MTS testing with GC
- Data support these MTS strips are acceptable for use in subsequent in vitro susceptibility testing.

References

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