Poster #701

Comparison of MIC Results for Gepotidacin by Agar Dilution and Broth Microdilution Methods

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Introduction

- Gepotidacin (GSK2140944) is a novel triazaacenaphthylene bacterial type II topoisomerase inhibitor in clinical development for the treatment of gonorrhea and uncomplicated urinary tract infection (acute cystitis)
- Gepotidacin selectively inhibits bacterial DNA gyrase and topoisomerase IV by a unique mechanism not utilized by any approved therapeutic agent and demonstrates *in vitro* activity against most target pathogens resistant to established antimicrobials, including fluoroquinolones
- The aim of this study, in accordance with Clinical and Laboratory Standards Institute (CLSI) M23-A4 guidelines (2016), was to test the equivalency of minimal inhibitory concentrations (MICs) obtained by 2 reference antimicrobial susceptibility testing methods, agar dilution and broth microdilution, for the investigational antimicrobial drug gepotidacin against various gram-positive and gram-negative organisms

Materials and Methods

- Susceptibility testing for both methods was performed on 733 clinical isolates recovered mostly in 2016 from over 120 medical centers worldwide
- MICs were determined by broth microdilution and agar dilution per CLSI M07-A10 methods (2015)
- Cation-adjusted Mueller-Hinton broth or Mueller-Hinton agar (MHA) was used for non-fastidious organisms
- Streptococci were tested in Mueller-Hinton broth supplemented with 2.5-5% lysed horse blood and MHA supplemented with 5% whole sheep blood
  - Most streptococci isolate broth microdilution MICs were measured after incubation in ambient air; however, broth microdilution MICs for *Streptococcus anginosus* group isolates were read after incubation in 5% CO_2
  - All streptococci agar MICs were determined with incubation in ambient air
  - *Haemophilus influenzae* isolate MICs were determined in *Haemophilus* test medium (HTM) for broth microdilution and agar dilution methods
- For *Neisseria gonorrhoeae*, agar dilution method using GC agar is the only recommended reference dilution method by CLSI; for comparison purposes, broth microdilution was performed using fastidious broth
- Broth microdilution and agar dilution for all species group isolates were performed on the same day from the same bacterial inoculum suspension
- Quality control (QC) strains were tested concomitantly with clinical isolates, and inoculum density was monitored by colony counts for the following QC strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *H. influenzae* ATCC 49247, *Streptococcus pneumoniae* ATCC 49619, and *N. gonorrhoeae* ATCC 49226
- Five ATCC QC isolates were evaluated with 20 replicate MIC results from at least 2 testing days and analyzed for intra- and inter-day reproducibility, including range, mean, mode, and standard deviation
- Essential agreement (EA) based on evaluable results was calculated as the number of isolates with MICs within one 2-fold dilution of the reference method divided by the total number of results
- Equivalency was defined using the 95% criteria from the Food and Drug Administration's class II controls document (2009)

Results

- Replicate testing against QC organisms demonstrated limited variability and no drug/isolate/MIC method combination had MIC values that differed by more than 1-$\log_2$ dilution from the mode (Table 1)
- For all isolates tested, MICs by these 2 methods had an EA of 85.8% (Table 2)
- A much greater agreement was seen when *H. influenzae* and *N. gonorrhoeae* isolates were excluded from the analysis (EA = 98.3%, Table 2 and Figure 1)
- Against *E. coli*, broth microdilution MICs for gepotidacin were often 1-$\log_2$ dilution higher than agar dilution MICs ($MIC_{50/90}$: 2/4 µg/mL, 1/2 µg/mL respectively, Table 3)
  - While the population is skewed for slightly higher MICs by broth microdilution, the EA with agar dilution is still high at 97.1% (Table 2)
- Among *Staphylococcus* spp., broth microdilution gepotidacin MICs were roughly equivalent to agar dilution MICs
  - The EA for *S. aureus* and *Staphylococcus saprophyticus* was 97.5% and 100%, respectively (Table 2)
- Among *S. pneumoniae* isolates tested, broth microdilution gepotidacin MICs were roughly equivalent to agar dilution MICs ($MIC_{50/90}$: 0.25/0.5 µg/mL; Table 3)
  - The EA for *S. pneumoniae* was 100.0% (Table 2)
- An EA of 100% was observed when testing gepotidacin against β-hemolytic streptococci isolates (Table 2), but the population was slightly skewed for higher gepotidacin MICs by broth microdilution
- Among viridans group streptococci isolates tested (including *Streptococcus anginosus* group isolates), overall broth microdilution MICs were roughly equivalent to agar MICs ($MIC_{50/90}$: 0.5/1 µg/mL; Table 3)
  - While the population was slightly skewed for higher gepotidacin MICs in broth microdilution, the EA with agar dilution was still high at 96.2% (Table 2)
- When testing against *H. influenzae* isolates, broth microdilution MICs were often 1-$\log_2$ dilution lower than agar dilution MICs for this species ($MIC_{50/90}$: 0.5/1 µg/mL, 1/2 µg/mL respectively; Table 3)
  - The EA for *H. influenzae* was lower than most other species at only 73.1% and only 11.5% had equivalent MICs by broth microdilution and agar dilution (Table 2)
- *N. gonorrhoeae* isolates displayed the greatest discrepancy between methods and broth microdilution MICs were often 2-$\log_2$ dilutions lower than agar dilution MICs for this species ($MIC_{50/90}$: 0.12/0.25 µg/mL, 0.5/1 µg/mL respectively; Table 3)
  - The EA for *N. gonorrhoeae* was lowest of all groups tested at only 28.6% and only 4.4% (Table 2) had equivalent MICs by broth microdilution and agar dilution
    - It is important to note that 106 *N. gonorrhoeae* isolates tested grew on GC agar, while only 91 of those grew in fastidious broth; therefore, EA was only able to be calculated on 91 isolates

Conclusions

- An EA of 98.3% was observed for *E. coli*, staphylococci, and streptococci species combined
- Slightly higher gepotidacin MICs, in most cases one two-fold dilution higher, were observed when tested by broth microdilution for each of these species/groups, and this trend was especially prominent for *E. coli* and *Streptococcus pyogenes*
- Poor EA was observed when testing *H. influenzae* and *N. gonorrhoeae* isolates (73.1% and 28.6%, respectively)
- With an EA of >95%, equivalency was established between the agar dilution and broth microdilution methods for determining susceptibility results for gepotidacin against *E. coli*, staphylococci, and streptococci species
- However, for *N. gonorrhoeae* and *H. influenzae*, equivalency between the 2 methods was not established; therefore, future antimicrobial susceptibility testing for gepotidacin against these organisms should adhere to the methods for which gepotidacin quality control ranges and breakpoints are approved

Table 1. Gepotidacin broth microdilution and agar dilution MIC results when tested against quality control strains *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, and *N. gonorrhoeae* ATCC 49226

Organism (no. of tests/no. of days)	MIC (µg/mL)			
Antimicrobial agent	Range	Mean	Standard deviation	Mode
***E. coli* ATCC 25922** (22/4)				
Gepotidacin agar dilution	1 – 2	1.2	0.429	1
Gepotidacin broth microdilution	1	1	0	1
***S. aureus* ATCC 29213** (22/4)				
Gepotidacin agar dilution	0.12 – 0.25	0.18	0.063	0.12
Gepotidacin broth microdilution	0.25	0.25	0	0.25
***S. pneumoniae* ATCC 49619** (20/3)				
Gepotidacin agar dilution	0.12 – 0.25	0.14	0.038	0.12
Gepotidacin broth microdilution	0.12 – 0.25	0.24	0.038	0.25
***H. influenzae* ATCC 49247** (22/2)				
Gepotidacin agar dilution	1	1	0	1
Gepotidacin broth microdilution	0.5	0.5	0	0.5
***N. gonorrhoeae* ATCC 49226** (23/2)				
Gepotidacin agar dilution	0.5	0.5	0	0.5
Gepotidacin broth microdilution	0.12	0.12	0	0.12

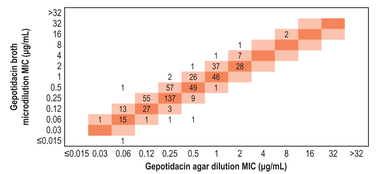
Table 2. Essential agreement between broth microdilution and agar dilution MICs for gepotidacin

Organism/organism group	% Essential agreement [b]	No. of isolates ($\log_2$ dilution difference)[a]						
		← Larger agar dilution MIC				Larger broth microdilution MIC →		
		≤-3	-2	-1	0	1	2	≥3
Gepotidacin								
All isolates	85.8	16	80	97	318	201	5	1
Isolates excluding *H. influenzae* and *N. gonorrhoeae*	98.3	1	2	14	302	198	5	1
E. coli	97.1				48	51	3	
Staphylococcus spp.	98.2			3	71	34	2	
S. aureus	97.5			2	53	23	2	
S. saprophyticus	100.0			1	18	11		
Streptococcus spp.	98.7	1	2	11	183	113		1
S. pneumoniae	100.0			4	79	18		
β-hemolytic streptococci	100.0				45	60		
S. pyogenes	100.0				27	50		
S. agalactiae	100.0				18	10		
Viridans group streptococci	96.2	1	2	7	59	35		1
S. anginosus	100.0			7	16	9		
H. influenzae	73.1	2	26	64	12			
N. gonorrhoeae	28.6	13	52	19	4	3		

[a] The $\log_2$ dilution difference calculated as the $\log_2$ (broth microdilution MIC value/agar dilution MIC value).

[b] Essential agreement calculated as the number of broth microdilution and agar dilution MIC values for each isolate that are ±1 $\log_2$ dilution (shaded values), divided by the total number of isolates.

Figure 1. Scattergram of gepotidacin broth microdilution MIC vs gepotidacin agar dilution MIC against all isolates, excluding *N. gonorrhoeae* and *H. influenzae* (n=523)



Dark orange shaded values represent instances with identical MIC values between methods.

Light orange shaded values represent instances of agreement included in essential agreement calculations (±1 $\log_2$ dilution).

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- NS-O is an employee of GlaxoSmithKline.
- MC was an employee of GlaxoSmithKline at the time of the study.

Table 3. Antimicrobial activity of gepotidacin tested by broth microdilution and agar dilution against the clinical isolates tested

Organism/test method (no. of isolates)	No. and cumulative % of isolates at MIC (µg/mL) of [a]:													MIC$_{50}$	MIC$_{90}$
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16			
E. coli (102)															
Broth microdilution method				0 0.0	1 1.0	2 2.9	29 31.4	59 89.2	8 97.1	1 98.0	2 100.0			2	4
Agar dilution method			0 0.0	1 1.0	2 2.9	10 12.7	51 62.7	36 98.0	0 98.0	2 100.0				1	2
Staphylococcus aureus (80)															
Broth microdilution method			0 0.0	5 6.2	44 61.2	28 96.2	3 100.0							0.25	0.5
Agar dilution method			0 0.0	8 10.0	60 85.0	12 100.0								0.25	0.5
S. saprophyticus (30)															
Broth microdilution method		0 0.0	15 50.0	15 100.0										0.06	0.12
Agar dilution method		0 0.0	25 83.3	5 100.0										0.06	0.12
Streptococcus pneumoniae (101)															
Broth microdilution method			0 0.0	9 8.9	66 74.3	26 100.0								0.25	0.5
Agar dilution method			0 0.0	12 11.9	74 85.1	15 100.0								0.25	0.5
S. pyogenes (77)															
Broth microdilution method			0 0.0	5 6.5	54 76.6	16 97.4	2 100.0							0.25	0.5
Agar dilution method			0 0.0	39 50.6	35 96.1	2 98.7	1 100.0							0.12	0.25
S. agalactiae (28)															
Broth microdilution method				0 0.0	1 3.6	6 25.0	16 82.1	5 100.0						1	2
Agar dilution method				0 0.0	1 3.6	11 42.9	16 100.0							1	1
Viridans group streptococci (105)															
Broth microdilution method	1 1.0	0 1.0	4 4.8	9 13.3	35 46.7	30 75.2	24 98.1	2 100.0						0.5	1
Agar dilution method	0 0.0	1 1.0	5 5.7	18 22.9	28 49.5	36 83.8	17 100.0							0.5	1
S. anginosus (32)															
Broth microdilution method		0 0.0	1 3.1	0 3.1	10 34.4	9 62.5	10 93.8	2 100.0						0.5	1
Agar dilution method	0 0.0	1 3.1	0 0.0	1 6.2	4 18.8	17 71.9	9 100.0							0.5	1
H. influenzae (104)															
Broth microdilution method		0 0.0	1 1.0	0 1.0	19 19.2	64 80.8	12 92.3	8 100.0						0.5	1
Agar dilution method				0 0.0	3 2.9	16 18.3	48 64.4	28 91.3	9 100.0					1	2
N. gonorrhoeae (106)															
Broth microdilution method [b]	3 3.3	5 8.8	17 27.5	52 84.6	12 97.8	1 98.9	1 100.0							0.12	0.25
Agar dilution method	0 0.0	2 1.9	4 5.7	18 22.6	24 45.3	46 88.7	12 100.0							0.5	1

[a] The intensity of shading is proportional to the % of tested isolates within each row that display the indicated MIC value.
[b] 15 *N. gonorrhoeae* isolates failed to grow in broth.

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