Poster # 2828

Comparison of MIC Results for Gepotidacin by Agar Dilution and Broth Microdilution Methods for Various Gram-negative and Gram-positive Species

S.J. Ryan Arends, PhD
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, IA 52317
Phone: (319) 665-3370
Fax: (319) 665-3371
Email: ryan-arends@jmilabs.com

SJR Arends[1], J. West[2], M. Horger[1], N. Scangarella-Oman[2], M. Castanheira[1]

[1] JMI Laboratories, North Liberty, Iowa; [2] GlaxoSmithKline, Collegeville, Pennsylvania

Introduction

- Gepotidacin (GSK2140944) is a novel, bactericidal, first in class triazaacenaphthylene antibiotic in clinical development for the treatment of gonorrhea and uncomplicated urinary tract infection (acute cystitis).
- Gepotidacin selectively inhibits bacterial DNA replication by a distinct mechanism of action which confers *in vitro* activity against most strains of target pathogens, such as *E. coli, S. saprophyticus* and *N. gonorrhoeae,* including those resistant to current antibiotics.
- The aim of this study, in accordance with Clinical and Laboratory Standards Institute (CLSI) M23-A4 guidelines (2018), 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 for 857 clinical isolates recovered from over 120 medical centers worldwide.
- All isolates were collected in 2020, except for 28 *Providencia rettgeri* isolates from 2019 due to lower prevalence.
- MICs were determined by broth microdilution and agar dilution per CLSI M07-A10 methods (2018).
- Cation-adjusted Mueller-Hinton broth or Mueller-Hinton agar (MHA) was used for all organisms.
- Broth microdilution and agar dilution for all isolates were performed on the same day from the same bacterial inoculum suspension.
- Due to the swarming nature of many *Proteus mirabilis* isolates, testing was carried out in 24-well, non-treated microtiter plates. A total volume of 600 µL of molten agar with the various drug concentrations was transferred into each well. After the agar was allowed to cool, each well was inoculated with a single isolate suspension at the appropriate concentration.
- 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, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213.
- Four ATCC QC isolates were evaluated with at least 21 replicate MIC results from at least 2 testing days and analyzed for intra- and interday 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. No drug/isolate/MIC method combination had MIC values that differed by more than 1-$\log_2$ dilution from the mode (Table 1).
- The modal gepotidacin MIC value for *E. faecalis* ATCC 29212 was 2-fold higher when determined by broth microdilution (2 µg/mL) than by agar dilution (1 µg/mL). Similarly, the modal gepotidacin MIC value for *S. aureus* ATCC 29213 was 2-fold higher when determined by broth microdilution (0.25 µg/mL) than by agar dilution (0.12 µg/mL; Table 1).
- When comparing all isolates, gepotidacin MIC values by these 2 methods showed good correlation (R^2 = 0.91) and an EA of 92.5% (Figure 1 and Table 2).
- For most species, broth microdilution MICs were often 1-$\log_2$ dilution higher than agar dilution MICs (Table 3).
- Against *E. faecalis*, broth microdilution $MIC_{50/90}$ 2/2 µg/mL and agar dilution $MIC_{50/90}$ 1/1 µg/mL were observed. The trend of higher MIC values determined by broth microdilution led to an EA of only 84.1% with the agar dilution method.
- Among *Staphylococcus saprophyticus* isolates, broth microdilution gepotidacin MICs were similar to agar dilution MICs ($MIC_{50/90}$: 0.06/0.12 µg/mL, 0.06/0.06 µg/mL respectively). The EA for *S. saprophyticus* was 90.7%.
- Against *Klebsiella* species isolates tested, EAs observed were 92.2% for all isolates, 95.4% for *K. pneumoniae*, 98.1% for *K. aerogenes*, but only 80.0% for *K. oxytoca.*
- For *Enterobacter cloacae* complex species and *Citrobacter* species, broth microdilution MIC values were often 1-$\log_2$ dilution higher than agar dilution MIC values. However, EAs observed were 95.3% for *E. cloacae* complex species, 94.3% for *C. freundii*, and 100.0% for *C. koseri.*
- Against *P. rettgeri*, broth microdilution $MIC_{50/90}$ 8/16 µg/mL and agar dilution $MIC_{50/90}$ 4/8 µg/mL were observed. The trend of higher MIC values determined by broth microdilution led to an EA of only 88.6% with the agar dilution method.
- Against *P. mirabilis*, broth microdilution gepotidacin $MIC_{50/90}$ values were equivalent to agar dilution MIC values for this species ($MIC_{50/90}$; 8/16 µg/mL) and all MIC values (100%) showed EA between broth microdilution and agar dilution.

Conclusions

- Limited intra- or interday variation was observed for MICs against the 4 QC strains and no isolate/testing method combination had MIC values that differed by more than 1-$\log_2$ dilution from the mode.
- An EA between broth microdilution and agar dilution methods of 92.5% was observed for all isolates tested.
- Slightly higher gepotidacin MICs, in most cases one 2-fold dilution higher, were observed when tested by broth microdilution for most species/groups tested.
- With an EA of >95%, equivalency was established between agar dilution and broth microdilution MIC methods for gepotidacin against *K. pneumoniae*, *K. aerogenes*, *E. cloacae* species complex, *P. mirabilis*, and *C. koseri.*
- Equivalency was not established between methods for *C. freundii* species complex, *S. saprophyticus*, *P. rettgeri*, *E. faecalis*, and *K. oxytoca.*

Table 1. Gepotidacin broth microdilution and agar dilution MIC results when tested against quality control strains *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 29213

Organism (no. of tests / no. of days)	MIC (µg/mL)			
Antimicrobial agent and testing method	Range	Mean	Standard Deviation	Mode
***E. coli* ATCC 25922**				
Gepotidacin agar dilution (21/9)[a]	1	1	0	1
Gepotidacin broth microdilution (24/12)	1 – 2	1.1	0.34	1
***E. faecalis* ATCC 29212**				
Gepotidacin agar dilution (21/9)	1	1	0	1
Gepotidacin broth microdilution (24/12)	1 – 4	2	0.51	2
***P. aeruginosa* ATCC 27853**				
Gepotidacin agar dilution (21/9)	8	8	0	8
Gepotidacin broth microdilution (24/12)	4 – 16	8.3	2.62	8
***S. aureus* ATCC 29213**				
Gepotidacin agar dilution (21/9)	0.12 – 0.25	0.13	0.04	0.12
Gepotidacin broth microdilution (24/12)	0.25	0.25	0	0.25

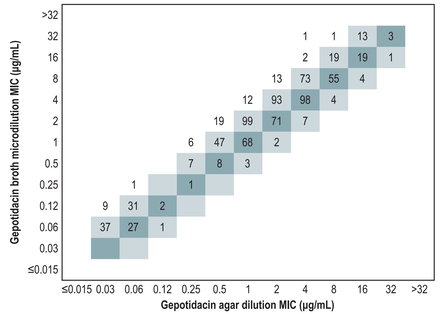
[a] MIC results from 24-well agar *P. mirabilis* testing not included in this analysis

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

		No. of isolates ($\log_2$ dilution difference)[a]							
		← Larger agar dilution MIC					Larger broth microdilution MIC →		
Organism/organism group	% Essential agreement[b]	≤-3	-2	-1	0	1	2	≥3
All isolates	92.5%			22	352	419	63	1	
Enterococcus faecalis	84.1%			1	20	69	17		
Staphylococcus saprophyticus	90.7%			1	29	68	10		
Klebsiella species	92.2%			1	93	107	17		
Klebsiella pneumoniae	**95.4%**[c]			1	45	58	5		
Klebsiella aerogenes	**98.1%**				30	23	1		
Klebsiella oxytoca	80.0%				18	26	11		
***Enterobacter cloacae* species complex**	**95.3%**			2	52	48	5		
Providencia rettgeri	88.6%			3	29	61	11	1	
Proteus mirabilis	**100.0%**			11	73	20			
***Citrobacter* species**	**97.2%**			3	56	46	3		
Citrobacter freundii species complex	94.3%			2	30	18	3		
Citrobacter koseri	**100.0%**			1	26	28			

[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 both microdilution and agar dilution MIC values for each isolate that are ±1 $\log_2$ dilution (shaded values), divided by the total number of isolates.
[c] Bold values denote essential agreement values >95%.

Figure 1. Scattergram of gepotidacin broth microdilution MIC vs gepotidacin agar dilution MIC against all isolates (n=857) with an essential agreement of 92.5%



Dark blue shaded values represent instances with identical MIC values between methods.
Light blue shaded values represent instances of agreement included in essential agreement calculations (±1 $\log_2$ dilution).

Acknowledgements/Disclosures

This study was supported by GlaxoSmithKline. JMI Laboratories received compensation for services related to preparing this poster.

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:												
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	MIC_{50}	MIC_{90}
Enterococcus faecalis (107)														
Broth microdilution method				0 0.0	1 0.9	4 4.7	30 32.7	68 96.3	3 99.1	1 100.0			2	2
Agar dilution method				0 0.0	6 5.6	31 34.6	60 90.7	8 98.1	2 100.0				1	1
Staphylococcus saprophyticus (108)														
Broth microdilution method		0 0.0	65 60.2	42 99.1	1 100.0								0.06	0.12
Agar dilution method	0 0.0	46 42.6	59 97.2	3 100.0									0.06	0.06
Klebsiella pneumoniae (109)														
Broth microdilution method					0 0.0	1 0.9	1 1.8	5 6.4	62 63.3	23 84.4	8 91.7	9 100.0	4	16
Agar dilution method				0 0.0	1 0.9	1 1.8	3 4.6	31 33.0	51 79.8	11 89.9	11 100.0		4	16
Klebsiella aerogenes (54)														
Broth microdilution method						0 0.0	6 11.1	38 81.5	10 100.0				2	4
Agar dilution method				0 0.0	1 1.9	0 1.9	20 38.9	31 96.3	2 100.0				2	2
Klebsiella oxytoca (55)														
Broth microdilution method						0 0.0	18 32.7	25 78.2	10 96.4	2 100.0			2	4
Agar dilution method				0 0.0	1 1.8	4 9.1	43 87.3	5 96.4	2 100.0				1	2
Enterobacter cloacae species complex (107)														
Broth microdilution method							0 0.0	29 27.1	53 76.6	12 87.9	9 96.3	4 100.0	4	16
Agar dilution method						0 0.0	7 6.5	53 56.1	32 86.0	8 93.5	5 98.1	2 100.0	2	8
Providencia rettgeri (105)														
Broth microdilution method						0 0.0	4 3.8	7 10.5	36 44.8	47 89.5	7 96.2	4 100.0	8	16
Agar dilution method					0 0.0	3 2.9	5 7.6	25 31.4	56 84.8	13 97.1	3 100.0		4	8
Proteus mirabilis (104)														
Broth microdilution method					0 0.0	1 1.0	4 4.8	5 9.6	21 29.8	56 83.7	16 99.0	1 100.0	8	16
Agar dilution method						0 0.0	4 3.8	9 12.5	28 39.4	45 82.7	16 98.1	2 100.0	8	16
Citrobacter freundii species complex (53)														
Broth microdilution method					0 0.0	5 9.4	24 54.7	14 81.1	8 96.2	1 98.1	1 100.0		1	4
Agar dilution method				0 0.0	2 3.8	14 30.2	18 64.2	13 88.7	4 96.2	1 98.1	1 100.0		1	4
Citrobacter koseri (55)														
Broth microdilution method					0 0.0	7 12.7	36 78.2	5 87.3	4 94.5	3 100.0			1	4
Agar dilution method				0 0.0	3 5.5	21 43.6	22 83.6	4 90.9	4 98.2	1 100.0			1	2

References

1. Clinical and Laboratory Standards Institute. *M07. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard.* 11th ed. Wayne, PA: CLSI; 2018.
2. Clinical and Laboratory Standards Institute. *M23. Development of in vitro susceptibility testing criteria and quality control parameters.* 5th ed. Wayne, PA: CLSI; 2018.
3. Clinical and Laboratory Standards Institute. *M100-Ed31. Performance standards for antimicrobial susceptibility testing: 27th informational supplement.* Wayne, PA: CLSI; 2021.
4. FDA. Guidance for industry and FDA. Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems. Rockville, MD, USA, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health; 2009.