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A Comparison of Broth Microdilution, Macrodilution and Agar Dilution for GSK2251052 and Meropenem against Gram-negative Organisms and *B. fragilis*

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Abstract

Background: GSK2251052 (formerly AN3365), a novel boron-containing leucyl-tRNA synthetase inhibitor with *in vitro* activity against *Pseudomonas aeruginosa* and multidrug-resistant Enterobacteriaceae, is currently being developed for the treatment of serious Gram-negative bacterial infections. This study was undertaken in order to compare broth microdilution, macrodilution and agar dilution methods for GSK2251052 and a comparator agent, meropenem. **Method:** 10 *E. coli*, 10 *K. pneumoniae*, 10 *Proteus* spp., 10 *P. aeruginosa*, 10 *B. fragilis* and eight quality control strains were tested by three CLSI susceptibility methods [broth microdilution (BMD), macrodilution (Macro) and agar dilution (AD)] and MICs compared. In addition, BMD MICs using two different broths [IsoSensitest (ISB) and brucella (BRU)] were compared to CLSI BMD MICs and AD MICs using IsoSensitest agar (ISA) were compared to CLSI AD MICs.
Results: GSK2251052 MICs for Enterobacteriaceae and *P. aeruginosa* were within ±1 dilution using the three CLSI testing methods. GSK2251052 AD MICs for *B. fragilis* were approximately 1.5 dilutions higher than BMD MICs. GSK2251052 BMD using ISB and BRU were approximately 2 and 1 dilutions higher, respectively, for Enterobacteriaceae and *P. aeruginosa* compared to CLSI BMD MICs. An increase in MICs was also observed for Enterobacteriaceae and *P. aeruginosa* by approximately 2 and 2.5 dilutions, respectively, when AD MICs using ISA were compared to CLSI AD MICs.

Mean dilution difference of GSK2251052 (GSK052) and meropenem (MER) MICs compared to CLSI BMD* MICs

Method	Enterobacteriacae		*P. aeruginosa*		*B. fragilis*	
	GSK052	MER	GSK052	MER	GSK052	MER
Macro	0.28	-0.06	0.03	0.17	N/A	N/A
AD	0.38	-0.23	-0.18	-0.08	**1.43**	0.08
BMD ISB	**2.06**	0.11	**2.00**	0.00	N/A	N/A
BMD BRU	**1.25**	0.67	0.88	-0.25	N/A	N/A
AD ISA*	**2.18**	-0.10	**2.48**	-0.17	N/A	N/A

*AD ISA results were compared to CLSI AD results
(bolded values are >± 1 dilution)

Conclusion: GSK2251052 MICs for Gram-negative aerobic organisms, using the three standard CLSI susceptibility testing methods, were within +/- one-half dilution. As is the case with other agents, GSK2251052 anaerobic agar dilution MICs can be slightly higher compared to BMD MICs. Consideration of potentially higher GSK2251052 MICs when using IsoSensitest agar and broth (e.g. BSAC methods) should be given in future *in vitro* susceptibility studies.

Introduction

- Susceptibility testing of antimicrobial agents is typically performed according to standardized methods, such as the widely utilized and accepted Clinical and Laboratory Standards Institute (CLSI) guidelines.
- These guidelines provide standardized procedures for controlling important testing conditions which have been shown to have an effect on susceptibility results.
- This study was undertaken in order to compare CLSI broth microdilution, macrodilution and agar dilution MIC methods and also compare broth and agar methods using different media for GSK2251052 and a comparator agent, meropenem.

Methods

Antibiotics (Concentrations):

•GSK2251052 (0.06 – 64 µg/mL)
•Meropenem (0.008 – 8 µg/mL)

Media:

•Cation Adjusted Mueller Hinton Broth (CAMHB) – Becton Dickinson, Sparks, MD
•Cation Adjusted Mueller Hinton Agar (MHA) – Becton Dickinson, Sparks, MD
•IsoSensitest Broth (ISB) – Oxoid Ltd., Ogdensburg NY
•IsoSensitest Agar (ISA) – Oxoid Ltd., Ogdensburg NY
•Brucella Broth (BRU) – Becton Dickinson, Sparks, MD
•Brucella Agar (BRUA) – Becton Dickinson, Sparks, MD (anaerobe testing only)

Methods

Microorganisms:

•11 *Escherichia coli*
•11 *Klebsiella pneumoniae*
• 7 *Proteus* spp.
•11 *Pseudomonas aeruginosa*
•10 *Bacteroides fragilis*

Quality Control Strains:
•*E. coli* ATCC 25922,
•*E. coli* ATCC 35218 (TEM-1 ß-lactamase)
•*P. aeruginosa* ATCC 27853
•*K. pneumoniae* ATCC 700603 (SHV-18 ESBL)
•*K. pneumoniae* ATCC BAA-1705 (KPC)
•*K. pneumoniae* ATCC BAA-1706 (resistant to carbapenem, not carbapenemase)
•*B. fragilis* ATCC 25285
•*B. thetaiotaomicron* ATCC 29741

MIC Methods:

MIC testing was performed according to the following CLSI procedures (with exception of broth other than CAMHB):

Broth Microdilution (BMD): CAMHB, ISB, BRU
Broth Macrodilution (MD): CAMHB
Agar Dilution (AD): MHA and ISA (BRUA for anaerobe testing only)

Triplicate testing was performed by all methods, utilizing the same inoculum

Results

Aerobic Organisms

Reproducibility of BMD (Figures 1 and 2):
GSK2251052 and meropenem mean MIC results, for 12 days of testing, were within ±1 doubling dilution for Enterobacteriaceae and *P. aeruginosa*.

Macrodilution Compared to BMD (Tables 1 and 2, Figure 3):
Essential agreement rate for GSK2251052 against Enterobacteriaceae and *P. aeruginosa* was 97.7% and 100%, respectively.

Essential agreement rates for meropenem against Enterobacteriaceae and *P. aeruginosa* were 100%.

Agar Dilution compared to BMD (Tables 1 and 2, Figure 3):
Essential agreement rate for GSK2251052 against Enterobacteriaceae and *P. aeruginosa* was 98.5% and 100%, respectively.

Essential agreement rates for meropenem against Enterobacteriaceae and *P. aeruginosa* were 100%.

Media Differences:

IsoSensitest broth and agar (Table 1 and 2, Figure 4):
GSK2251052 MIC results were at least 2 doubling dilutions higher using ISB and ISA compared to CAMHB and MHA, respectively, against both Enterobacteriaceae and *P. aeruginosa*. In contrast, meropenem MIC results were not affected by ISB and ISA against both Enterobacteriaceae and *P. aeruginosa*.

Brucella broth (Table 1 and 2, Figure 4):
GSK2251052 and meropenem MIC results were increased by approximately one doubling dilution with the use of BRU against Enterobacteriaceae. GSK2251052 MIC results were also approximately one doubling dilution higher and meropenem MIC results were similar with the use of BRU against *P. aeruginosa*.

Anaerobic Organisms (BMD compared to AD):

For *B. fragilis*, GSK2251052 AD MIC results were 1.43 dilution higher overall compared to BMD MIC results. The meropenem AD MIC results were similar to the BMD MIC results (Table 3).

Table 1. *In vitro* activity of GSK2251052 and meropenem for 29 Enterobacteriaceae as determined by broth microdilution, macrodilution and agar dilution methodologies

Test (CLSI Reference) Method	Comparative Method	n	GSK2251052 Mean MICs (mcg/mL)	GSK2251052 Mean MIC difference[a]	GSK2251052 Mean Dilution Difference[b]	GSK2251052 N(%) ±1 dilution[c]	GSK2251052 N(%) ±2 dilution[c]	n	Meropenem Mean MICs (mcg/mL)	Meropenem Mean MIC difference[a]	Meropenem Mean Dilution Difference[b]	Meropenem N(%) ±1 dilution[c]	Meropenem N (%) ±2 dilution[c]
Broth Microdilution - CAMHB (BMD)	Macrodilution - CAMHB (MD)	87	BMD= 0.83 MD= 1.01	0.18	0.28	85 (97.7%)	87 (100%)	69	BMD= 0.06 MD= 0.06	0.00	-0.06	66 (95.7%)	69 (100%)
Broth Microdilution - CAMHB (BMD)	Agar Dilution –MHA (MH-AD)	66	BMD= 0.83 MH-AD= 1.02	0.19	0.38	65 (98.5%)	66 (100%)	48	BMD= 0.06 MH-AD= 0.04	-0.03	-0.23	48 (100%)	48 (100%)
Agar Dilution –MHA (MH-AD)	Agar Dilution -ISA (IS-AD)	66	MH-AD= 1.02 IS-AD= 4.63	3.61	2.18	3 (0.05%)	51 (77.3%)	48	MHA= 0.04 ISA= 0.03	0.00	-0.10	46 (95.8%)	48 (100%)
Broth Microdilution - CAMHB (BMD)	Broth Microdilution - ISB	87	BMD= 0.83 ISB= 3.47	2.63	2.06	5 (0.06%)	77 (88.5%)	69	BMD= 0.06 ISB= 0.07	0.01	0.12	66 (95.7%)	66 (95.7%)
Broth Microdilution - CAMHB (BMD)	Broth Microdilution - BRU	87	BMD= 0.83 BRU= 1.98	-0.83	1.25	64 (73.6%)	86 (98.9%)	69	BMD= 0.06 BRU= 0.10	0.04	0.69	56 (81.2%)	60 (87.0%)

a. Difference in log_2 Mean MICs, Comparative Method – Reference Method
b. Dilution differences were calculated for each MIC by subtracting the log_2+10 test MIC from the log_2+10 reference MIC and mean dilution differences were determined for each method.
c. Number and percentage of MICs by the 2 methods within ±1 doubling dilutions (essential agreement) or ±2 doubling dilutions of each other

Table 2. *In vitro* activity of GSK2251052 and meropenem for 11 *P. aeruginosa* as determined by broth microdilution, macrodilution and agar dilution methodologies

Test (CLSI Reference) Method	Comparative Method	n	GSK2251052 Mean MICs (mcg/mL)	GSK2251052 Mean MIC difference[a]	GSK2251052 Mean Dilution Difference[b]	GSK2251052 N(%) ±1 dilution[c]	GSK2251052 N(%) ±2 dilution[c]	n	Meropenem Mean MICs (mcg/mL)	Meropenem Mean MIC difference[a]	Meropenem Mean Dilution Difference[b]	Meropenem N(%) ±1 dilution[c]	Meropenem N (%) ±2 dilution[c]
Broth Microdilution - CAMHB (BMD)	Macrodilution - CAMHB (MD)	33	BMD= 4.00 MD= 4.08	0.08	0.03	33 (100%)	33 (100%)	12	BMD= 0.75 MD= 0.84	0.09	0.17	12 (100%)	12 (100%)
Broth Microdilution - CAMHB (BMD)	Agar Dilution -MHA (MH-AD)	33	BMD= 4.00 MH-AD= 3.53	-0.47	-0.18	33 (100%)	33 (100%)	12	BMD= 0.75 MH-AD= 0.71	-0.04	-0.08	12 (100%)	12 (100%)
Agar Dilution –MHA (MH-AD)	Agar Dilution -ISA (IS-AD)	33	MH-AD= 3.53 IS-AD= 19.74	16.21	2.48	1 (0.03%)	19 (57.6%)	12	MHA= 0.71 ISA= 0.63	-0.08	-0.17	12 (100%)	12 (100%)
Broth Microdilution - CAMHB (BMD)	Broth Microdilution - ISB	33	BMD= 4.00 ISB= 14.99	10.99	2.00	2 (0.06%)	30 (90.9%)	12	BMD= 0.75 ISB= 0.75	0.00	0.00	12 (100%)	12 (100%)
Broth Microdilution - CAMHB (BMD)	Broth Microdilution - BRU	33	BMD= 4.00 BRU= 7.36	3.36	0.88	32 (97.0%)	33 (100%)	12	BMD= 0.75 BRU= 0.63	-0.12	-0.25	12 (100%)	12 (100%)

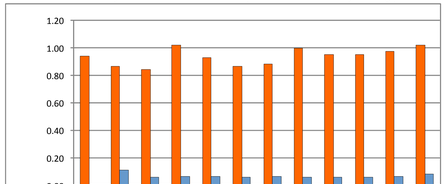
a. Difference in log_2 Mean MICs, Comparative Method – Reference Method
b. Dilution differences were calculated for each MIC by subtracting the log_2+10 test MIC from the log_2+10 reference MIC and mean dilution differences were determined for each method.
c. Number and percentage of MICs by the 2 methods within ±1 doubling dilutions (essential agreement) or ±2 doubling dilutions of each other

Table 3. *In vitro* activity of GSK2251052 and meropenem for 10 *B. fragilis* as determined by broth microdilution and agar dilution methodologies

Test (CLSI Reference) Method	Comparative Method	GSK2251052 n	GSK2251052 Mean MICs (mcg/mL)	GSK2251052 Mean MIC difference[a]	GSK2251052 Mean Dilution Difference[b]	GSK2251052 N(%) ±1 dilution[c]	GSK2251052 N(%) ±2 dilution[c]	Meropenem n	Meropenem Mean MICs (mcg/mL)	Meropenem Mean MIC difference[a]	Meropenem Mean Dilution Difference[b]	Meropenem N(%) ±1 dilution[c]	Meropenem N(%) ±2 dilution[c]
Broth Microdilution (BMD)	Agar Dilution (AD)	30	BMD= 2.41 AD= 6.50	4.09	1.43	17 (56.7%)	30 (100%)	24[d]	BMD= 1.11 AD= 1.08	0.07	0.08	24 (100%)	24 (100%)

a. Difference in log_2 Mean MICs, Comparative Method – Reference Method
b. Dilution differences were calculated for each MIC by subtracting the log_2+10 test MIC from the log_2+10 reference MIC and mean dilution differences were determined for each method.
c. Number and percentage of MICs by the 2 methods within ±1 doubling dilutions (essential agreement) or ±2 doubling dilutions of each other
d. Two strains were not included in the dilution difference analysis because MICs were off-scale (>16 mcg/ml)

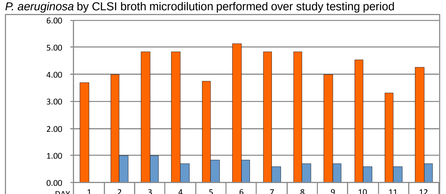
Figure 1. Geometric mean MICs (mcg/mL) of GSK2251052 and meropenem for 29 Enterobacteriaceae by CLSI broth microdilution performed over study testing period



DAY	1	2	3	4	5	6	7	8	9	10	11	12
GSK2251052	0.94	0.87	0.85	1.02	0.93	0.87	0.89	1.00	0.95	0.95	0.98	1.02
Meropenem		0.11	0.06	0.07	0.07	0.06	0.07	0.06	0.06	0.06	0.07	0.09

GSK2251052 Meropenem

Figure 2. Geometric mean MICs (mcg/mL) of GSK2251052 and meropenem for 11 *P. aeruginosa* by CLSI broth microdilution performed over study testing period



DAY	1	2	3	4	5	6	7	8	9	10	11	12
GSK2251052	3.70	4.00	4.83	4.83	3.76	5.15	4.83	4.83	4.00	4.54	3.31	4.26
Meropenem		1.00	1.00	0.71	0.84	0.84	0.59	0.71	0.71	0.59	0.59	0.71

GSK2251052 Meropenem

Figure 3. Geometric mean MICs (mcg/mL) of GSK2251052 by broth microdilution, broth macrodilution and agar dilution

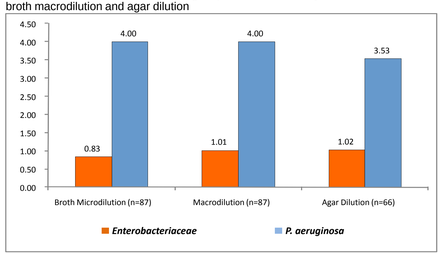
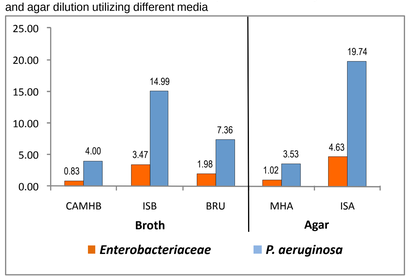


Figure 4. Geometric mean MICs (mcg/mL) of GSK2251052 by broth microdilution and agar dilution utilizing different media



Conclusions

- The GSK2251052 MIC results using CLSI BMD were very reproducible over twelve days of testing (mean MIC results varied no more than 0.17 doubling dilution).
- The GSK2251052 macrodilution and agar dilution MIC results were within 1 doubling dilution of the broth microdilution MIC results for all study isolates.
- Enterobacteriaceae and *P. aeruginosa* GSK2251052 BMD and AD MIC results using IsoSensitest media were 2 doubling dilutions higher compared to CAMHB and MHA, respectively. IsoSensitest media is used by the British Society of Antimicrobial Chemotherapy.
- *B. fragilis* GSK2251052 AD MIC results were approximately 1½ doubling dilutions higher compared to BMD MIC results.

Acknowledgements

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