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Multilaboratory Broth Microdilution MIC Reproducibility Study for GSK3342830, a Novel Catechol-Cephem

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Abstract

Background: GSK3342830 (GSK830) is a novel catechol-cephem antibiotic being developed to treat infections caused by gram-negative pathogens, including those with multidrug-resistant phenotypes. GSK830 has enhanced β-lactamase stability and contains a catechol group that facilitates entry into the bacterial cell via iron uptake mechanisms. The *in vitro* activity of GSK830 is affected by iron in the medium. As free iron is limited in humans, iron limited testing conditions may be more reflective of the *in vivo* state. The aim of this study was to determine a susceptibility (S) media testing condition resulting in acceptable GSK830 MIC reproducibility.

Methods: Reference *in vitro* S testing methods were used to evaluate the reproducibility of GSK830 MIC values against 22 bacterial strains (*Enterobacteriaceae* [EB], *Pseudomonas aeruginosa* [PA], and *Acinetobacter baumannii* [AB]). Testing comprised 5 laboratories, 5 replicates per isolate, using 5 separate inocula over a minimum of 3 days on microtiter panels produced by Thermo Fisher Scientific in cation-adjusted Mueller-Hinton broth (CAMHB); CAMHB with apo-transferrin (apo-T, 1.6 mg/mL; 20μM); iron-depleted, Chelex®-treated CAMHB (ID-CAMHB); Iso-Sensitest™ broth (ISB); ISB with apo-T (1.6 mg/mL; 20μM); and Chelex-treated ISB. MIC values were recorded as the first well with no visible growth (100% inhibition); 80% inhibition of growth was also recorded if different than the 100% endpoint.

Results: GSK830 showed acceptable inter- and intra-laboratory MIC reproducibility against most *Escherichia coli*, *Klebsiella pneumoniae* (KP), PA, and AB in CAMHB or ID-CAMHB with approximately 80% of strains having ≤4 different MIC results, all within a 4-dilution range. Apo-T treated medium exhibited the greatest variability in results. MIC values were much lower for some isolates in iron-depleted media (IDM). The largest differences were seen for KP, which exhibited lower MIC values in IDM (apo-T treated medium providing the largest differences) where MIC values ranged to as much as 7 dilutions lower in CAMHB-apo-T compared to CAMHB. Significant trailing growth occurred for 1 AB isolate (less pronounced in ISB). MIC interpretation (100% endpoint) and an 80% endpoint if trailing occurred provided a potential method for obtaining consistent GSK830 MIC results. CAMHB and ID-CAMHB provided more reproducible MIC results than apo-T-treated medium.

Conclusions: GSK830 has demonstrated acceptable MIC reproducibility when tested in CAMHB or ID-CAMHB, which facilitates progression for antimicrobial susceptibility testing.

Introduction

- GSK3342830 is a novel catechol-cephem antibiotic with enhanced stability to β-lactamases. It inhibits bacterial cell wall synthesis and contains a catechol group on the 3-position side chain which is believed to facilitate entry into the bacterial cell via iron uptake mechanisms. GSK3342830 is being developed for the treatment of infections caused by gram-negative bacteria including multidrug resistant (MDR) isolates for when there are limited or no other therapeutic options available.
- *The sp*ectrum of activity for GSK3342830 contains *Enterobacteriaceae,* including multi-drug resistant isolates of *Klebsiella pneumoniae, Escherichia coli,* and non-fermenting bacteria (*Pseudomonas aeruginosa* and *Acinetobacter baumannii)*
- GSK3342830 *in vitro* activity is affected by iron in the medium; therefore, MIC values are generally reduced for gram-negative organisms when tested in iron-depleted media
- The purpose of this study was to evaluate multiple media types to identify a testing medium that would provide reproducible MIC values for GSK3342830

Materials and Methods

- A total of 22 bacterial isolates, including ATCC quality control strains, were tested (Table 1)

Table 1 Bacterial isolates tested against GSK3342830

Organism (no.)	Strain	Resistance profile/genotype
E. coli (5)	ATCC 25922	QC strain
	NCTC 13441	CTX-M-15; Uropathogenic strain O25:H4; ST-131
	5649	NDM-1; CMY-30; OXA-1; TEM-1
	IR5	NDM-1; CTX-M-15; CTX-M-1
	ALL	NDM-1; CTX-M-15; OXA-1; OXA-2; TEM-1
K. pneumoniae (7)	ATCC 13883	QC strain
	ATCC 700603	SHV18, KPC
	NCTC 13443	NDM-1; CTX-M-15; CMY-4; DHA; TEM
	VA-384	KPC-2; SHV-12; SHV-14; TEM-1
	VA-391	KPC-3; SHV-12; SHV-77; TEM-1
	6560-MAR	NDM-1; CTX-M-15; OXA-1; OXA-9; SHV-5; TEM-1
	PLE	OXA-48
A. baumannii (5)	ATCC 19606	QC strain
	1484815	MDR
	1485247	MDR; OXA-51; ADC
	1485283	MDR; OXA-51; ADC; OXA-24
	1485176	MDR; OXA-51; ADC-30
P. aeruginosa (5)	ATCC 27853	QC strain
	SR27001	MDR; IMP-1
	SBRKM-28	MDR; IMP-1; VIM-2
	1484185	MDR; IMP-1
	SR24888	MDR; IMP-1

- Broth microdilution panels were produced by a commercial manufacturer (Thermo Fisher Scientific, Cleveland , Ohio), frozen, and shipped to each participant site
  - Six medium types were tested: cation-adjusted Mueller-Hinton broth (CAMHB), CAMHB with apo-T (1.6 mg/mL), Chelex®-treated CAMHB (CT-CAMHB), Iso-Sensitest broth (ISB), ISB with apo-T (1.6 mg/mL), and CT-ISB
    - Chelex-treated media and media containing apo-T are considered iron-depleted media
  - ISB conditions were included for *A. baumannii* testing only
- Panels were inoculated and incubated following CLSI recommended procedures (M07-A10; Clinical and Laboratory Standards institute, 2015) for *E. coli, K. pneumoniae, P. aeruginosa*, and *A. baumannii*
  - 5 replicates per isolate, using 5 separate inocula, were tested over a minimum of 3 days
- After incubation, panels were read to determine the minimal inhibitory concentration (MIC) as the first well with no visible growth of the pathogen
  - Laboratories were instructed to record both 100% inhibition of growth and 80% inhibition of growth if different
- Testing was performed in 5 participant laboratories

Investigator	Laboratory and Location
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C. Knapp, M.S.	Thermo Fisher Scientific, Cleveland, OH
M. Zervos, M.D.	Henry Ford Hospital, Detroit, MI
E. Munson, Ph.D.	Wheaton Franciscan Laboratory, Wauwatosa, WI
R. Rennie, Ph.D.	University of Alberta Hospitals, Edmonton, Alberta, Canada

- Replicate MIC results from the 5 sites were compiled, the overall modal MIC value for each strain was determined, and the intra- and inter-site reproducibility was described as the percentage of MIC results that were within +/- 1 dilution or +/- 2 dilutions of the overall mode

Results

- All-laboratory modal MIC values from replicate testing were identified for each of the 22 isolates in CAMHB, apo-T CAMHB, and CT-CAMHB (Table 2)
  - MIC values for GSK3342830 were typically highest in CAMHB compared to apo-T or CT-CAMHB
  - MIC values for GSK3342830 were typically lower in apo-T-treated media than in Chelex-treated media
  - For *A. baumannii*, MIC values for GSK3342830 were slightly lower in ISB than in CAMHB for all 3 media condition comparisons, except for 1 isolate (1485247) that was >4-fold lower

Table 2 Summary of the overall modal MIC values (mg/L) for 22 isolates tested against GSK3342830 using 2 media types under 3 testing conditions when read at 100% and 80% inhibition of growth

		Cation-adjusted Mueller-Hinton broth						Iso-Sensitest broth					
		Standard		apo-T supplemented		Chelex treated		Standard		apo-T supplemented		Chelex treated	
Organism	ID#	100%	80%	100%	80%	100%	80%	100%	80%	100%	80%	100%	80%
E. coli	ATCC 25922	0.12	0.06	0.12	0.12	0.25	0.12	0.25	0.25	0.03	0.03	0.12	0.12
E. coli	NCTC 13441	0.25	0.25	0.12	0.06	0.25	0.25						
E. coli	5649	32	32	4	4	8	8						
E. coli	IR5	64	64	8	4	16	8						
E. coli	ALL	8	8	4	4	8	8						
K. pneumoniae	ATCC 13883	0.25	0.25	0.03	0.03	0.12	0.12						
K. pneumoniae	ATCC 700603	0.25	0.06	0.015	0.015	0.12	0.12						
K. pneumoniae	NCTC 13443	16	16	0.12	0.12	4	2						
K. pneumoniae	VA-384	4	4	0.06	0.06	2	1						
K. pneumoniae	VA-391	0.5	1	0.03	0.03	1	0.5						
K. pneumoniae	6560-MAR	1	1	0.5	0.5	0.5	0.5						
K. pneumoniae	PLE	0.03	0.03	0.015	0.008	0.03	0.03						
A. baumannii	ATCC 19606	0.25	0.25	0.12	0.06	0.12	0.06	0.25	0.12	0.06	0.03	0.12	0.12
A. baumannii	1484815	2	1	0.5	0.5	0.5	0.25	2	2	0.25	0.06	0.25	0.25
A. baumannii	1485247	>64	4	>64	4	>64	1	4	2	2	0.5	2	1
A. baumannii	1485283	1	0.5	0.25	0.25	0.25	0.12	0.5	0.5	0.12	0.12	0.25	0.25
A. baumannii	1485176	0.25	0.12	0.25	0.12	0.12	0.06	0.12	0.12	0.12	0.06	0.06	0.06
P. aeruginosa	ATCC 27853	1	0.5	0.5	0.5	0.5	0.25	1	1	0.5	0.5	0.5	0.5
P. aeruginosa	SR27001	32	32	1	1	8	4						
P. aeruginosa	SBRKM-28	0.5	0.25	0.12	0.12	0.25	0.12						
P. aeruginosa	1484185	1	0.5	1	0.5	0.5	0.5						
P. aeruginosa	SR24888	0.5	0.25	1	1	0.25	0.25						

- For each media type, GSK3342830 MIC values (100% or 80% inhibition varied less for *E. coli, K. pneumoniae*, and *P. aeruginosa* isolates compared to *A. baumannii* (Table 2)
  - Most *A. baumannii* isolates demonstrated a 1-dilution lower MIC value when read at 80% compared to 100%, which was most prominent in CT-CAMHB
  - One isolate, *A. baumannii* (1485247), showed a greater than 16- to 32-fold increase in MIC when read at 100% in all 3 CAMHB media variations
- When testing in CAMHB, ≥90% of MIC values for 17 isolates were +/- 1 dilution of the overall mode, and ≥95% of MIC values for 19 isolates were +/- 2 dilutions of the overall mode (Table 4)
- For testing in CT-CAMHB, ≥90% of MIC values for 15 isolates were at +/- 1 dilution and ≥95% of MIC values for 22 isolates were +/- 2 dilutions of the overall mode
- Apo-T treated medium showed the least MIC reproducibility with only 12 isolates showing ≥90% of MIC values within +/- 1 dilution of the overall mode and 14 isolates with ≥95% of MIC values +/- 2 dilutions of the overall mode
- Overall for the 22 isolates, the CT-CAMHB all-laboratory modal MICs were the same or within 1 dilution of the overall CAMHB modal MIC values for 14 isolates and within 2 dilutions for the remaining 8 isolates (Table 2)
- Under apo-T conditions, the modal MICs for 12/22 isolates were the same or within 1 dilution of the overall CAMHB modal MIC values, 2 isolates were 2 dilutions lower, 3 isolates were 3 dilutions lower, 2 isolates were 4 dilutions lower, and there was 1 each at 5, 6, and 7 dilutions lower
- For *E. coli* tested in CAMHB, 4 isolates exhibited 92-96% agreement (+/- 1 dilution of the all-laboratory modal MIC) and 1 isolate exhibited 80% agreement (Table 3)
  - Variability was similar in apo-T- and Chelex-treated medium except for 1 isolate, 5649, where there was better MIC reproducibility with CT-CAMHB
- More variability was seen when testing *K. pneumoniae*, which appeared to be less pronounced in Chelex-treated medium than in apo-T-treated medium (Table 3)
  - A total of 6 of 7 isolates exhibited MIC values that were +/- 1 dilution of the mode less than 90% of the time (60–88%) in apo-T compared to 3 of 7 isolates in Chelex-treated medium at less than 90% (84–88%)
  - In Chelex-treated medium, all 7 *K. pneumoniae* isolates provided MIC results that were within +/- 2 dilutions of the all-laboratory mode 95–100% of the time compared to the results with apo-T where 5 of 7 isolates only exhibited 75–84% of MIC values within 2 dilutions of the modal MIC (Table 3)
  - MIC values for 6 of 7 isolates were all within 2 dilutions of the all-laboratory modal MIC when testing in CAMHB

Table 3 Summary of the modal MIC values and the percentage of results for an individual isolate when testing GSK3342830 within the 5 testing sites to fall within +/- 1 dilution or +/- 2 dilutions of the overall mode

		Mode GSK3342830 MIC (all labs)			% MICs +/- 1 dilution of mode MIC			% MICs +/- 2 dilutions of mode MIC		
Organism	Isolate	CAMHB	apo-T	Chelex	CAMHB	apo-T	Chelex	CAMHB	apo-T	Chelex
E. coli[a]	ATCC 25922	0.12	0.12	0.25	92	92	100	100	100	100
	NCTC 13441	0.25	0.12	0.25	96	100	100	100	100	100
	5649	32	4	8	80	80	84	80	80	100
	IR5	64	8	16	96	96	96	96	96	96
	ALL	8	4	8	96	96	96	96	96	96
All *E. coli*					92.0	92.8	95.2	94.4	94.4	98.4
K. pneumoniae[a]	ATCC 13883	0.25	0.03	0.12	92	80	84	100	80	100
	ATCC 700603[b]	0.25	0.015	0.12	65	75	90	100	75	95
	NCTC 13443	16	0.12	4	84	60	88	100	76	96
	VA-384	4	0.06	2	100	80	100	100	84	100
	VA-391	0.5	0.03	1	64	80	100	80	80	100
	6560-MAR	1	0.5	0.5	100	96	88	100	100	96
	PLE	0.03	0.015	0.03	92	88	100	100	96	100
All *K. pneumoniae*					85.9	80.0	92.9	97.1	84.7	98.2
A. baumannii[a]	ATCC 19606	0.25	0.06	0.06	100	96	100	100	100	100
	1485247	4	4	1	100	60	60[c]	100	80	96[c]
	1485283	0.5	0.25	0.12	100	100	100	100	100	100
	1485176	0.12	0.12	0.06	100	100	96	100	100	100
All *A. baumannii*					100.0	84.8	88.0	100.0	94.4	99.2
P. aeruginosa[a]	ATCC 27853	1	0.5	0.5	96	100	96	100	100	100
	SR27001	32	1	8	100	100	100	100	100	100
	SBRKM-28	0.5	0.12	0.25	100	92	100	100	100	100
	1484185	1	1	0.5	72	100	76	92	100	100
	SR24888	0.5	1	0.25	92	88	100	100	100	100
All *P. aeruginosa*					92.0	96.0	94.4	98.4	100.0	100.0
All isolates combined					91.9	87.5	92.7	97.4	92.6	98.9

[a] 100% inhibition endpoint in ID-MHB or CAMHB as MIC data used for analysis for all *E. coli, K. pneumoniae, P. aeruginosa*; 80% inhibition endpoint in ID-MHB or CAMHB used for analysis for all *Acinetobacter* isolates
[b] Only 20 results for this isolate
[c] Based on a median MIC of 1 mg/L

- All *A. baumannii* MIC values were within +/- 1 dilution of the overall mode when using 80% inhibition as the endpoint in CAMHB (Table 3)
  - A total of 60–100% of MIC values were within +/- 1 dilution in iron-depleted CAMHB, and 80–100% were within +/- 2 dilutions of the all-laboratory mode (Table 3)

Conclusions

- GSK3342830 showed good inter- and intra-laboratory reproducibility when tested against most isolates of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* in CAMHB or CT-CAMHB
- Susceptibility testing with apo-T supplemented CAMHB provided the least reproducible MIC values
- The differences between MIC values when testing in CAMHB and iron-depleted medium for *E. coli* and *P. aeruginosa* were less pronounced than those observed for *K. pneumoniae*
- All *K. pneumoniae* isolates exhibited lower MIC values in iron-depleted media with apo-T-treated medium providing the larger differences
- For *A. baumannii*, reading at the 80% endpoint provided reproducible MIC results for all 5 isolates tested in both CAMHB and ISB with or without iron depletion

Table 4 Summary of GSK3342830 MIC reproducibility according to media type

Testing medium (broth)	Number of isolates with ≤4 MIC results—all within 3 dilutions (total n=22)	Number of isolates with ≥90% MIC values +/- 1 dilution of overall laboratory mode MIC (total n=22)	Number of isolates with ≥95% MIC values +/- 2 dilutions of overall laboratory mode MIC (total n=22)
CAMHB	17	17	19
CAMHB + apo-T	13	12	14
CAMHB Chelex treated	18	15	22

CAMHB, cation-adjusted Mueller-Hinton broth

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