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Proposed MIC Quality Control Ranges for CEM-101 Using the CLSI Multi-Laboratory M23-A2 Study Design

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ABSTRACT

Background: CEM-101 is a promising new macrolide-ketolide in development for treating community-acquired (CA) macrolide-resistant and -susceptible bacteria. This study was performed to establish quality control (QC) ranges for CEM-101 for use in clinical or reference laboratories when performing Clinical and Laboratory Standards Institute (CLSI) broth microdilution MIC methods. QC strains included *S. aureus* ATCC 29213 (SA), *E. faecalis* ATCC 29212 (EF), *S. pneumoniae* ATCC 49619 (SPN) and *H. influenzae* ATCC 49247 (HI).

Methods: CLSI broth microdilution methods were utilized in an eight laboratory study design compliant with M23-A2 specifications. Four media lots (three manufacturers) of cation-adjusted Mueller-Hinton (MH) broth (with 2-5% lysed horse blood for testing SPN) or HTM broth were evaluated. Ten replicate MIC tests were performed for each QC organism generating 320 values for each strain (1,280 total). Azithromycin and/or erythromycin and/or clarithromycin were used as internal controls.

Results: The table lists the recommended MIC QC ranges for CEM-101. Modal MIC values (% of total) observed were: SA at 0.06 µg/ml (64.1), EF at 0.03 µg/ml (67.8), SPN at 0.008 µg/ml (85.3) and HI at 2 µg/ml (93.1). No significant differences were noted between media lots or testing site performance for either CEM-101 or the three control agents. All control agent MIC values were within CLSI published ranges.

	CEM-101 MIC (µg/ml)	
QC Organism (ATCC no.)	Proposed range (log_2 dilutions)	% in range
S. aureus ATCC 29213	0.03 – 0.12 (3)	96.6
E. faecalis ATCC 29212	0.015 – 0.06 (3)	95.6
S. pneumoniae ATCC 49619	0.004-0.015 (3)	99.3
H. influenzae ATCC 49247	1 – 4 (3)	99.7

Conclusions: CEM-101 is a novel macrolide-ketolide to be directed against CA respiratory tract infections and possibly other infections commonly treated with MLS_B-class agents. Proposed MIC QC ranges will help guide clinical or reference laboratories involved in the testing of clinical trial isolates and facilitate the regulatory review process.

INTRODUCTION

CEM-101 is an investigational macrolide-ketolide class antimicrobial agent with spectrum features superior to existing macrolides and most similar to telithromycin, although two- to four-fold more active. This broth microdilution quality control (QC) study of CEM-101 follows the NCCLS M23-A2 (2001) guideline document using eight laboratories, 4 lots/3 manufacturers of media and antimicrobial control agents. The results are presented as proposed QC ranges in µg/ml concentrations for four ATCC strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49619 and *Haemophilus influenzae* ATCC 49247).

MATERIALS AND METHODS

A total of eight laboratories were recruited to provide sufficient data for this QC investigation. Four cation-adjusted Mueller-Hinton (MH) broth media included lots produced by Difco Laboratories (Detroit, MI), Becton Dickinson (BD Sparks, MD), and Oxoid (Hampshire, United Kingdom). Four cation-adjusted MH broth lots supplemented with 2-5% lysed horse blood and four lots of Haemophilus Test Media (HTM) were also supplied by Difco, BD and Oxoid. CEM-101 was provided by Cempra Pharmaceuticals, Inc. (Chapel Hill, NC); azithromycin, erythromycin and clarithromycin were acquired from Sigma-Aldrich (St. Louis, MO). Panels were prepared by a certified GMP source, (TREK Diagnostics Cleveland, OH).

Internal QC was established using erythromycin for *S. aureus* and *E. faecalis* as a "peer drug" comparator agent and azithromycin and clarithromycin for *S. pneumoniae* and *H. influenzae* testing. Appropriate inoculum concentrations were established by performing colony counts from the broth microdilution trays which were subcultured onto drug-free agar plates. The average colony counts among the participating centers ranged from 2.6 X 10^5 CFU/ml to 5.7 X 10^5 CFU/ml.

RESULTS

- The CEM-101 MIC results from eight laboratories for *S. aureus* ATCC 29213 are shown in Table 1 and Figure 1. Using M23 criteria to establish MIC ranges, 96.6% of all results were within the proposed limits of 0.03 – 0.12 µg/ml (modal MIC ± one log_2 dilution step).
- *E. faecalis* ATCC 29212 MIC results are shown in Table 1 and Figure 2 with 95.6% of all results within the proposed limits of 0.015 – 0.06 µg/ml. All of the out-of-range MIC results were from one laboratory (Laboratory G).
- Figure 3 shows *S. pneumoniae* ATCC 49619 results with a proposed range of 0.004 – 0.015 µg/ml. A high percentage of all MICs (99.3%) were within this range with 85.3% of all CEM-101 MIC results at the modal MIC of 0.008 µg/ml.
- A very narrow CEM-101 MIC distribution is presented in Figure 4 for *H. influenzae* ATCC 49247 with a proposed QC range of 1 – 4 µg/ml. The MIC mode at 2 µg/ml represents 93.1% of all results reported.
- There was no significant difference between lots of Mueller-Hinton broth when testing CEM-101. The modal CEM-101 MIC was the same for all lots regardless of QC strain tested (0.06 µg/ml for *S. aureus*, 0.03 µg/ml for *E. faecalis*, 0.008 µg/ml for *S. pneumoniae,* and 2 µg/ml for *H. influenzae*), see Figures 1-4.

Table 1. Inter- and intra-laboratory comparisons of CEM-101 MIC results versus four ATCC control strains for an eight laboratory protocol conforming to the study design guidelines found in NCCLS M23-A2 (2001).

		Laboratory code (occurrences):								
Organism	MIC (µg/ml)	A	B	C	D	E	F	G	H	Total
S. aureus ATCC 29213	0.03									0[a]
	0.06	25	24	21	36	34	31	18	16	205[a]
	0.12	15	16	16	0	6	9	18	24	104[a]
	0.25			3	4			4		11
	Total	40	40	40	40	40	40	40	40	320
	Mode	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.12	0.06
	Range	2	2	3	3	2	2	3	2	3
E. faecalis ATCC 29212	0.015					10	2		2	14[b]
	0.03	39	12	20	40	30	38	4	34	217[b]
	0.06	1	28	20				22	4	75[b]
	0.12							14		14
	Total	40	40	40	40	40	40	40	40	320
	Mode	0.03	0.06	0.03	0.03	0.03	0.03	0.06	0.03	0.03
	Range	2	2	2	1	2	2	3	3	4
S. pneumoniae ATCC 49619	≤0.002			2						2
	0.004		7	25					4	36[c]
	0.008	35	33	13	40	40	40	37	35	273[c]
	0.015	5						3	1	9[c]
	Total	40	40	40	40	40	40	40	40	320
	Mode	0.008	0.008	0.004	0.008	0.008	0.008	0.008	0.008	0.008
	Range	2	2	3	1	1	1	2	3	4
H. influenzae ATCC 49247	0.5			1						1
	1			20						20[d]
	2	40	40	19	40	40	40	39	40	298[d]
	4							1		1[d]
	Total	40	40	40	40	40	40	40	40	320
	Mode	2	2	1	2	2	2	2	2	2
	Range	1	1	3	1	1	1	2	1	4

a. 96.6% of qualified results in proposed QC range (0.03 – 0.12 µg/ml).
b. 95.6% of qualified results in proposed QC range (0.015 – 0.06 µg/ml).
c. 99.3% of qualified results in proposed QC range (0.004 – 0.015 µg/ml).
d. 99.7% of qualified results in proposed QC range (1 – 4 µg/ml).

Figure 1. CEM-101 MIC distribution for *S. aureus* ATCC 29213

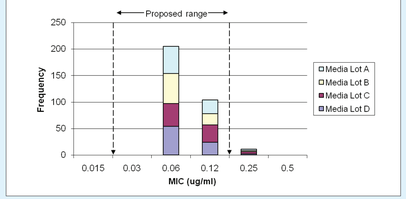


Figure 2. CEM-101 MIC distribution for *E. faecalis* ATCC 29212

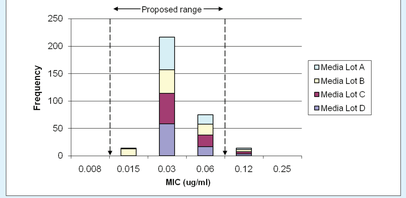


Figure 3. CEM-101 MIC distribution for *S. pneumoniae* ATCC 49619

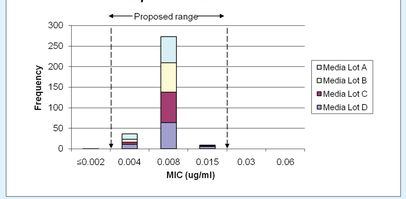
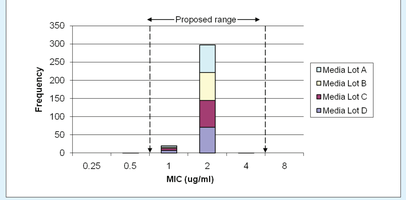


Figure 4. CEM-101 MIC distribution for *H. influenzae* ATCC 49247



CONCLUSIONS

- This study established QC ranges that can be utilized to support accurate testing for susceptibility of CEM-101 during clinical trials and continued product development.
- CEM-101 is a novel macrolide-ketolide to be directed against community-aquired respiratory tract infections and possibly other infections commonly treated with MLS_B-ketolide class agents.
- The proposed QC ranges provided in this study show that laboratories can accurately test CEM-101 with excellent inter- and intra-laboratory reproducibility for the commonly utilized control isolates, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, and *H. influenzae* ATCC 49247.

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