D-0706

Recommended Ulifloxacin MIC Quality Control Ranges Using a CLSI Multi-Laboratory M23-A2 Study Design

DJ BIEDENBACH, TR FRITSCHE, JE ROSS, RN JONES

JMI Laboratories, North Liberty, IA, USA

ICAAC 2006
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370,
fax 319.665.3371
ronald-jones@jmilabs.com

ABSTRACT

Background: Ulifloxacin is the active form of the orally administered prodrug prulifloxacin that possess a long half-life and a broad-spectrum of antimicrobial activity which includes enteropathogenic bacterial species. This study was performed to establish credible quality control ranges for ulifloxacin for use in clinical laboratories when performing reference broth microdilution MIC methods. Quality control (QC) strains included *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. jejuni* ATCC 33560 (CJ).

Methods: This study utilized CLSI broth microdilution methods with an eight laboratory study design compliant with M23-A2 specifications. Four media lots (three manufacturers) of cation-adjusted Mueller-Hinton (MH) broth were evaluated; 2-5% lysed horse blood was added for testing of CJ. Ten MIC replicates were performed with each of the three QC organisms, generating 320 values for each strain (960 total values). Ciprofloxacin was used as the internal control agent.

Results: The following table lists the recommended/proposed QC ranges for ulifloxacin:

	Ulifloxacin MIC (µg/ml)	
QC Organism	Proposed range (dilutions)	% in range
E. coli ATCC 25922	0.004 – 0.016 (3)	100.0
P. aeruginosa ATCC 27853	0.12 – 0.5 (3)	100.0
C. jejuni ATCC 33560	0.5 - 4 (4)	100.0[a]

a. Excluding Laboratory E, seven sites total (92.5% of results were in range if Laboratory E was included in the analysis).

Variability in MIC results between MH lots was not observed and only one center had results that were skewed towards lower MIC values for one QC strain (CJ).

Conclusions: Prulifloxacin is a potent oral fluoroquinolone currently approved for clinical use in some European countries and in Japan. Pharmacokinetic/pharmacodynamic indices suggest that the agent could be useful in the treatment of bacterial gastroenteritis. In this study, we propose MIC ranges for three QC strains that should assist clinical laboratories in providing accurate ulifloxacin susceptibility test results in support of ongoing clinical trials worldwide.

INTRODUCTION

Ulifloxacin is a broad-spectrum fluoroquinolone which is administered orally as a prodrug formulation (prulifloxacin). Esterases metabolize prulifloxacin to ulifloxacin upon absorption and this antimicrobial agent has positive pharmacological properties including excellent pharmacodynamic target attainment, long-elimination half-life (once daily dosing) and good tolerability. These factors, as well as excellent in vitro potency, have established this agent as a treatment option for numerous clinical indications and pathogens including: staphylococci, streptococci, enterococci and enteric or non-enteric Gram-negative bacilli. Clinical trials have shown that ulifloxacin is efficacious for the treatment of acute uncomplicated and complicated urinary tract infections as well as respiratory tract infections. Clinical application against gastroenteritis is under investigation. Tentative minimum inhibitory concentration (MIC) and disk diffusion breakpoints have been proposed with MIC criteria identical to that of ciprofloxacin.

This investigation provides MIC quality control (QC) range guidelines for ulifloxacin and three clinically important enteric and non-enteric Gram-negative bacilli. A multicenter study using Clinical and Laboratory Standards Institute (CLSI) methods adhering to the M23-A2 study design was performed to determine the appropriate QC ranges for three American Type Culture Collection (ATCC) strains which included: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Campylobacter jejuni* ATCC 33560.

MATERIALS AND METHODS

A total of eight laboratories were recruited to provide sufficient data for this QC investigation. A total of four broth media lots were tested for both the fastidious (*C. jejuni*) and non-fastidious (*E. coli* and *P. aeruginosa*) QC strains. 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 were supplied by BD and Difco. Ulifloxacin was provided by Optimer Pharmaceuticals, Inc. (San Diego, CA); ciprofloxacin was acquired from Sigma-Aldrich (St. Louis, MO). Panels were prepared by a certified GMP source, TREK Diagnostics (Cleveland, OH).

Internal QC was established using ciprofloxacin as a "peer drug" comparator agent. 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 3.1 X 10^5 CFU/ml to 4.1 X 10^5 CFU/ml.

RESULTS

- Table 1 provides the inter- and intra-laboratory results for all three QC strains tested against ulifloxacin. Against *E. coli* ATCC 25922, 81.3% of all eight laboratories' results were at the MIC mode (0.008 µg/ml) with most of the remaining results at the next highest log_2 dilution step (0.016 µg/ml). Nearly all results (97.0%) when testing *P. aeruginosa* ATCC 29853 were at the modal value of 0.25 µg/ml. Variability between laboratories was not significant when testing either of these control strains.
- When testing *C. jejuni* ATCC 33560, more variability was noted with both inter- and intra-laboratory results (Table 1). Only 49.4% of the results were at the MIC mode (1 µg/ml) and 37.8% at 2 µg/ml. One laboratory's results were skewed toward lower MIC values 0.25-0.5 µg/ml.
- Table 2 shows the distribution of MIC values obtained with each of the four media lots. In general, media lots performed similarly.
- The proposed ulifloxacin QC ranges for *E. coli* ATCC 25922 (Figure 1a) of 0.004-0.016 µg/ml and *P. aeruginosa* ATCC 27853 (Figure 1b) of 0.12-0.25 µg/ml included 100.0% of the participant test results. The four dilution MIC QC range for *C. jejuni* ATCC 33560 (Figure 1c) of 0.5-4 µg/ml included 92.5% of the results with Laboratory E data included and all (100.0%) results when excluding this laboratory's data.
- All internal QC data results for ciprofloxacin were within the CLSI (M100-S16, 2006) MIC QC ranges for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27053; and 99.4% for *C. jejuni* ATCC 33560.

Table 1. Inter- and intra-laboratory comparisons of ulifloxacin MIC results versus three 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
E. coli ATCC 25922	0.004				3				3	6[a]
	0.008	38	34	31	31	31	26	35	34	260[a]
	0.016	2	6	9	6	9	14	5	3	54[a]
	Total	40	40	40	40	40	40	40	40	320
	Mode	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
	Range	2	2	2	3	2	2	2	3	3
P. aeruginosa ATCC 27853	0.12	2								2[b]
	0.25	38	40	39	40	40	39	37	40	313[b]
	0.5			1			1	3		5[b]
	Total	40	40	40	40	40	40	40	40	320
	Mode	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Range	2	1	2	1	1	2	2	1	2
C. jejuni ATCC 33560	0.25					24				0/24
	0.5					16			1	1/17[c]
	1	40	21	20	20		18		39	158/158[c]
	2		19	20	20		22	40		121/121[c]
	4									0/0[c]
	Total	40	40	40	40	40	40	40	40	320
	Mode	1	1	1	1	0.25	2	2	1	1
	Range	1	2	2	2	2	2	1	2	4

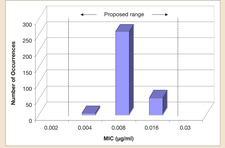
a. 100% of qualified results in proposed QC range (0.004 – 0.016 µg/ml).
b. 100% of qualified results in proposed QC range (0.12 – 0.5 µg/ml).
c. 100% of qualified results in proposed QC range (0.5 – 4 µg/ml), excluding Laboratory E . A total of 92.5% were in the proposed range when using results from all eight sites.

Table 2. Comparison of the ulifloxacin MIC distributions among four lots of Mueller-Hinton broth when testing three ATCC control strains.

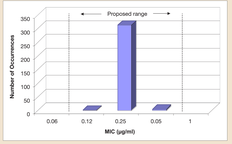
		Occurrences by lot:				
Organism	MIC (µg/ml)	A	B	C	D	Total
E. coli ATCC 25922						
	0.004	2	2	2		6
	0.008	75	75	63	47	260
	0.016	3	3	15	33	54
	Total	80	80	80	80	320
	Mode	0.008	0.008	0.008	0.008	0.008
	Range	3	3	3	2	3
P. aeruginosa ATCC 27853						
	0.12			2		2
	0.25	80	80	77	76	313
	0.5			1	4	5
	Total	80	80	80	80	320
	Mode	0.25	0.25	0.25	0.25	0.25
	Range	1	1	3	2	3
C. jejuni ATCC 33560						
	0.25	6	6	6	6	24
	0.5	4	4	4	5	17
	1	51	53	28	26	158
	2	19	17	42	43	121
	4	0	0	0	0	0
	Total	80	80	80	80	320
	Mode	1	1	2	2	1
	Range	4	4	4	4	4

Figure 1. Proposed ulifloxacin MIC quality control range for testing:

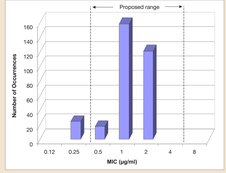
a. *E. coli* ATCC 25922;



b. *P. aeruginosa* ATCC 27853;



c. *C. jejuni* ATCC 33560.



CONCLUSIONS

- Prulifloxacin is a potent orally administered fluoroquinolone prodrug with potential utility for the treatment of bacterial gastroenteritis, among other common clinical indications.
- This study established QC ranges that can be utilized to support accurate testing for susceptibility of prulifloxacin during clinical trials.
- The proposed QC ranges provided in this report show that laboratories can accurately test ulifloxacin with excellent inter- and intra-laboratory reproducibility for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Although testing *C. jejuni* ATCC 33560 was slightly more problematic due to the fastidious nature of this organism, a credible range could also be established.

ACKNOWLEDGEMENTS

Participating laboratories and principle investigators utilized for the determination of ulifloxacin QC ranges included T.R. Fritsche (JMI Laboratories), C. Knapp (TREK Diagnostics), R. Rennie (University of Alberta), B. Reller (Duke University Medical Center), A. Wanger (University of Texas Medical School at Houston), D. Hardy (University of Rochester Medical Center), J. Meng (University of Maryland) and S. Bodeis-Jones (FDA/CVM).

SELECTED REFERENCES

Clinical and Laboratory Standards Institute. (2006). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. Approved Standard M7-A7*. Wayne, PA: CLSI, 2006.

Clinical and Laboratory Standards Institute. (2006). *Performance standards for antimicrobial susceptibility testing, 16th informational supplement M100-S16*. Wayne, PA: CLSI.

Keam SJ, Perry CM (2004). Prulifloxacin. *Drugs* 64: 2221-2234; discussion 2235-2226.

National Committee for Clinical Laboratory Standards. (2001). *Approved guideline M23-A2: Development of in vitro susceptibility testing criteria and quality controls parameters, 2nd ed*. Wayne, PA: NCCLS.

Ozaki M, Matsuda M, Tomii Y, Kimura K, Kazuno K, Kitano M, Kise M, Shibata K, Otsuki M, Nishino T (1991). In vitro antibacterial activity of a new quinolone, NM394. *Antimicrob Agents Chemother* 35: 2490-2495.

Ozaki M, Matsuda M, Tomii Y, Kimura K, Segawa J, Kitano M, Kise M, Shibata K, Otsuki M, Nishino T (1991). In vivo evaluation of NM441, a new thiazeto-quinoline derivative. *Antimicrob Agents Chemother* 35: 2496-2499.

Yoshida T, Mitsuhashi S (1993). Antibacterial activity of NM394, the active form of prodrug NM441, a new quinolone. *Antimicrob Agents Chemother* 37: 793-800.