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Proposed Disk Diffusion Quality Control Ranges for Ulifloxacin (Active Metabolite of Prulifloxacin) and Intermethod Comparison Using CLSI Methods

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AMENDED ABSTRACT

Background: Ulifloxacin (ULI; active metabolite of the prodrug prulifloxacin) is a broadly-active fluoroquinolone (FQ) displaying a favorable pharmacokinetic/pharmacodynamic profile that makes it an excellent choice for community-acquired infections such as traveler's diarrhea (TD) and urinary tract infections. We propose disk diffusion (DD) quality control (QC) ranges and tentative epidemiologic breakpoints (BPT) based upon CLSI methodologies.

Methods: ULI DD QC ranges were established per a CLSI M23-A2 study design. Ten replicates with each of 3 QC strains produced 1,440 zone diameters for ULI (5-µg disk). Broth microdilution MIC/DD regression analysis using error rate bounding was also performed for 562 TD pathogens (*E. coli* [EC], *Salmonella, Shigella, Yersinia, Aeromonas* [AER], *Plesiomonas, Campylobacter* [CPY] and *Vibrio* spp.). Ciprofloxacin (CIP) was the control agent.

Results: Proposed QC ranges are listed in the Table. No significant differences (>1mm) were noted between media or disk lots when testing either ULI or CIP. Using ULI MIC BPTs of ≤1 (susceptible [S]) and ≥4 µg/ml (resistant [R]; equivalent to CIP), and DD BPTs of ≤15 (R) and ≥19 mm (S; Montanari, J Chemother 17:138, 2005), total error was minimized to 0.7% (two EC, one each CPY and AER), similar to CIP (0.7%).

QC organism	Proposed range (mm)	% of results in range
S. aureus ATCC 25923	20 – 26	99.0[a]
E. coli ATCC 25922	32 – 38	97.9
P. aeruginosa ATCC 27853	27 – 33	95.6

a. Excluding one of eight laboratories; minimum participants required (≥7) was achieved.

Conclusions: Prulifloxacin (ULI, active metabolite) is presently marketed in certain European countries and Japan, and is in late-stage USA clinical trials for TD. Proposed DD QC ranges will help guide laboratories involved in testing of clinical trial isolates and facilitate regulatory review. Tentative DD BPTs correlate well with MIC values (≤1 µg/ml), producing minimal categorical errors (0.7%) and should prove useful for patient management and in monitoring for R emergence.

INTRODUCTION

Traveler's diarrhea (TD) is the most common ailment affecting persons visiting developing regions. Gastroenteritis also remains common in countries that have established sanitation infrastructure. Bacteria such as toxigenic and invasive strains of *Escherichia coli*, *Campylobacter spp.*, *Salmonella spp.*, *Shigella spp.*, *Vibrio spp.*, *Aeromonas spp.* and *Pleisiomonas shigelloides* are among the most common causes of bacterial gastrointestinal infections including those associated with TD. Symptoms can be mild or severe, and are especially problematic among the older, younger and immunocomprised patient groups. First-line antimicrobial agents for the treatment of TD include fluoroquinolones.

Ulifoxacin, the active metabolite of the oral fluoroquinolone prulifloxacin, has been evaluated in clinical trials for the treatment of infectious diarrhea. This agent has potent in vitro activity against a variety of pathogens including Enterobacteriaceae and nonfermentative Gram-negative bacilli, as well as Gram-positive organisms including *Staphylococcus* spp. and *Enterococcus* spp. Currently, ulifloxacin is being marketed in selected European countries and Japan.

The purpose of this study was to establish disk diffusion quality control (QC) ranges for ulifloxacin for three American Type Culture Collection (ATCC) strains (*S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) using the results from an eight laboratory study. Secondly, this study tested a large number of isolates to propose correlative breakpoint criteria for MIC and disk diffusion values by performing an inter-method comparison of ulifloxacin on gastroenteritis-producing pathogens. Regression lines were calculated, correlation coefficients determined and error rates were calculated for proposed breakpoint criteria.

MATERIALS AND METHODS

Ulifloxacin disk diffusion QC ranges were established using the Clinical and Laboratory Standards Institute (CLSI) M23-A2 study design. The eight participating laboratories used three different Mueller-Hinton agar lots (BBL, Sparks, MD, USA and Remel, Lenexa, KS, USA) and two different ulifloxacin disk lots (Oxoid, Cambridge, UK). Ten replicates of each of the three ATCC strains produced 1,440 zone diameters for ulifloxacin. Concurrent testing of gentamicin and ciprofloxacin were used as control agents.

Broth microdilution MIC and disk diffusion tests for the regression analysis were performed as recommended by the CLSI M7-A7 (2006) and M2-A9 (2006) methods, respectively. A collection of 562 gastrointestinal pathogens were selected, mostly from patients with documented gastroenteritis and from stool and bloodstream cultures. The species tested included *E. coli* (100 strains), *Salmonella* spp. (103), *Shigella* spp. (99), *Yersinia* spp. (43), *Aeromonas* spp. (101), *Plesiomonas* spp. (1), *Campylobacter* spp. (104), and *Vibrio* spp. (11); all were tested against ulifloxacin and ciprofloxacin (control agent). QC strains were tested concurrently and all results were within published ranges (CLSI M100-S17, 2007).

RESULTS

- Only 7 laboratories were used for determining a disk diffusion QC range for *S. aureus* ATCC 25923 due to a potential technical error discovered at one of the laboratories (Laboratory F). A proposed disk diffusion zone range of 20-26 mm for *S. aureus* resulted in 99.0% of test results within the proposed range (Figure 1).

Figure 1. Ulifloxacin zone diameter distributions for *S. aureus* ATCC 25923.

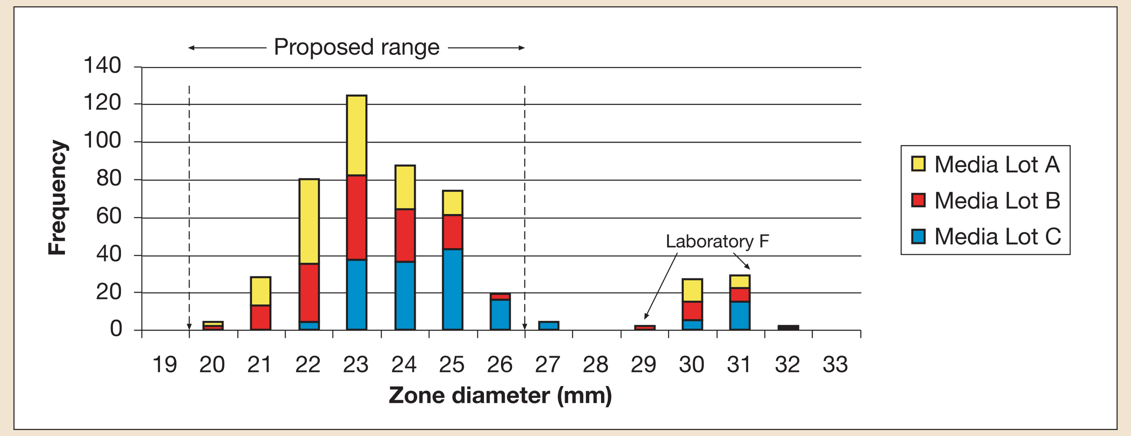


Figure 2. Ulifloxacin zone diameter distributions for *E. coli* ATCC 25922.

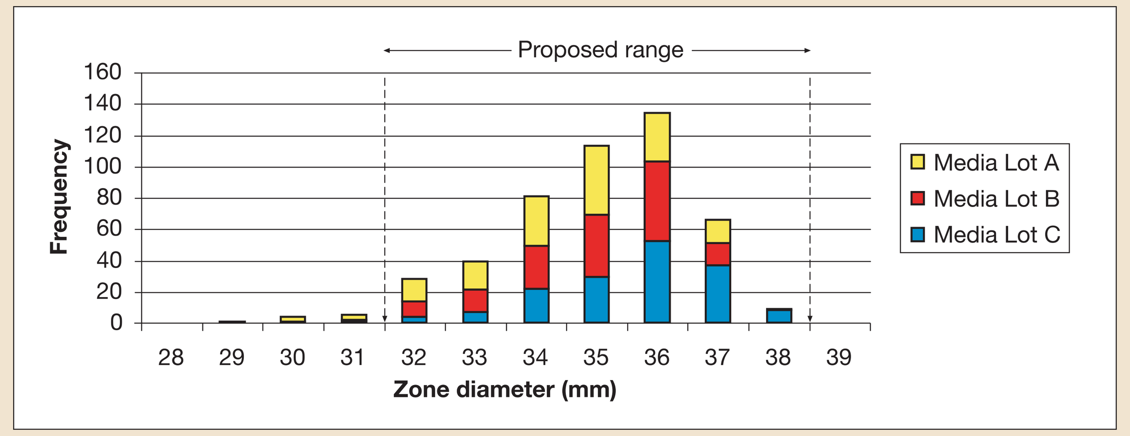
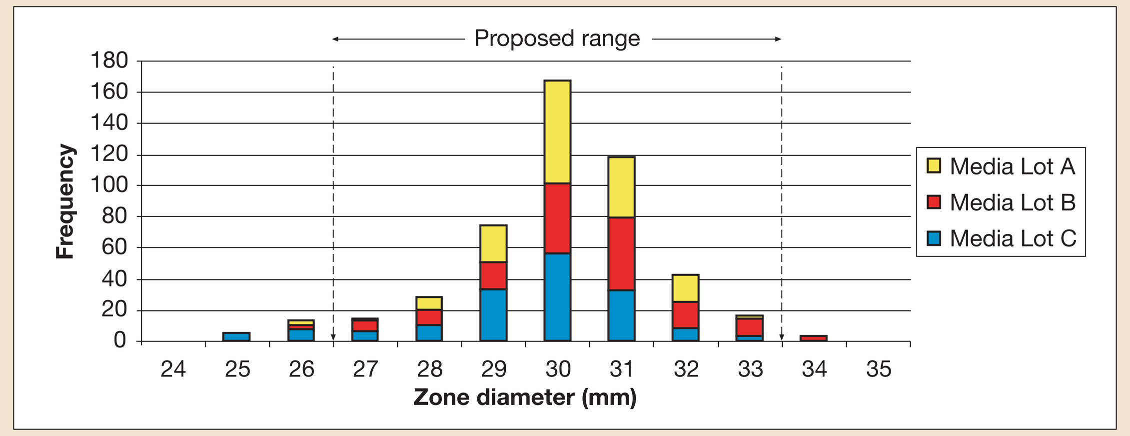
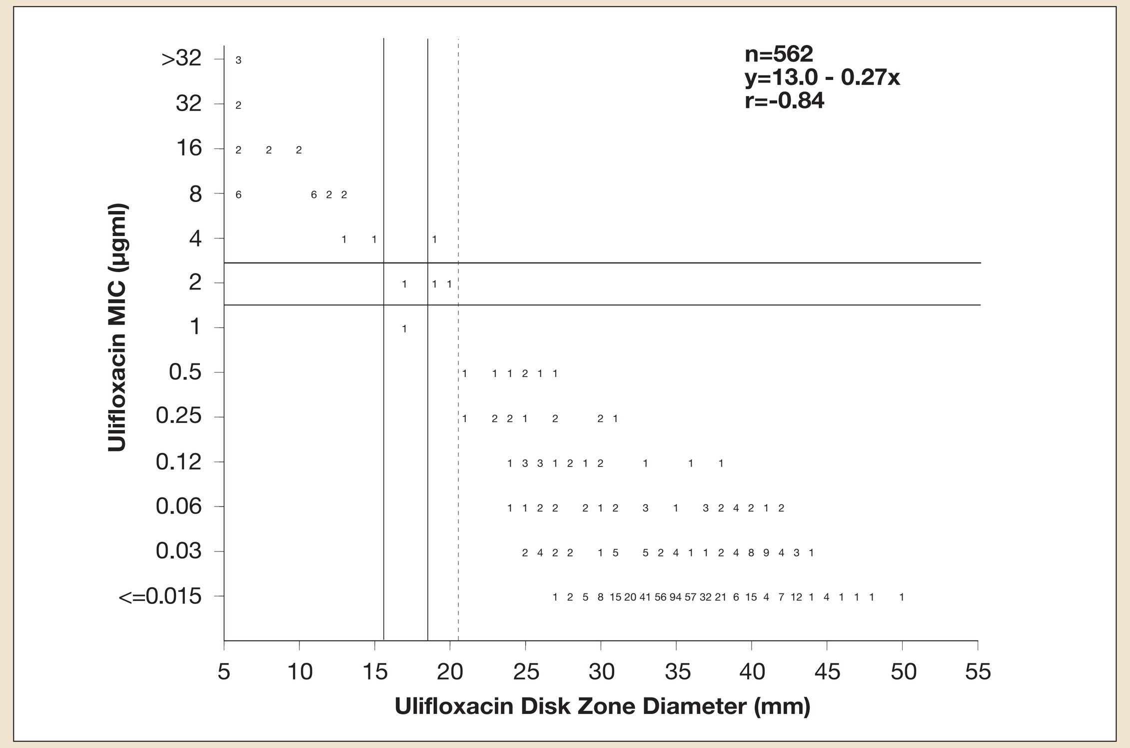


Figure 3. Ulifloxacin zone diameter distributions for *P. aeruginosa* ATCC 27853.



- Eight laboratories were used to establish ranges for the other ATCC strains. The proposed ranges for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were 32-38 mm (97.9% of results in range [Figure 2]) and 27-33 mm (95.6% of results in range [Figure 3]), respectively.
- No significant discords were observed between lots of media or ulifloxacin disks with all median zone diameters showing no more than 1 mm variation.
- Ulifloxacin inhibited 94.1% of the 562 gastroenteritis-producing organisms tested, identical to that of ciprofloxacin. Ulifloxacin- and ciprofloxacin-resistant strains were only detected among *E. coli* (17 and 19%, respectively), *Campylobacter spp.* (both 11.5%) and *Aeromonas spp.* (both 1%). All other strains tested were susceptible to ulifloxacin and ciprofloxacin (≤0.5 µg/ml for ulifloxacin and ≤1 µg/ml for ciprofloxacin).
- Figure 4 shows that the tentative disk diffusion breakpoints for Enterobacteriaceae, as published by Montanari et al. (≤15 mm as resistant [R] and ≥19 mm as susceptible [S]) correlated extremely well with proposed published MIC values (≤1/≥4 µg/ml for S/R) producing very low categorical error rates (0.7%). Utilization of breakpoints identical to those of ciprofloxacin (≤15 mm [R], 16-20 mm [intermediate] and ≥21 mm [S]) further reduces inter-method error to 0.4% (99.6% categorical agreement).

Figure 4. Regression analysis of ulifloxacin MIC values versus disk (5-µg) zone diameters for 562 gastroenteritis pathogens.



CONCLUSIONS

- Ulifloxacin, the active compound of prodrug prulifloxacin, is very potent against the most common bacterial pathogens causing gastroenteritis and displayed activity equal to that of ciprofloxacin.
- The proposed disk diffusion breakpoint criteria (2 options) correlated well with MIC breakpoint values (≤1/≥4 µg/ml) and adoption of either set of tentative disk diffusion breakpoints for ulifloxacin should prove useful for patient management and in monitoring for resistance emergence.
- The results from the ulifloxacin QC study and the QC ranges proposed should provide confidence for the laboratory when testing this potent fluoroquinolone in clinical practice.

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