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In Vitro Activity of Ulifloxacin (Active Metabolite of Prulifloxacin) Tested Against a Worldwide Collection of Pathogens Producing Traveler's Diarrhea

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AMENDED ABSTRACT

Background: Ulifloxacin (ULI; active metabolite of prodrug prulifloxacin) is a fluoroquinolone (FQ) with broad activity against enteric and non-enteric bacilli including enteropathogenic species. A long half-life, recognized safety profile and tissue penetration, make ULI an excellent choice for urinary tract infections and bacterial gastroenteritis. We tested ULI for activity against 8 pathogen groups known to cause traveler's diarrhea (TD).

Methods: 582 gastroenteritis strains from global surveillance studies were susceptibility tested against ULI, ciprofloxacin (CIP), rifaximin (RXM), ampicillin (AMP) and trimethoprim/sulfamethoxazole (SXT) using the CLSI broth microdilution method (see Table). *E. coli* (EC) types included EHEC (21), EPEC (20), ETEC (20), EaggEC (19) and bloodstream isolates (20).

Organism (no. tested)	MIC_{90} (µg/ml)				
	ULI	CIP	RXM	AMP	SXT
E. coli (EC; 100)	0.008	0.016	16	>256	>64
Aeromonas spp. (AER; 101)	0.06	0.06	8	>256	8
Campylobacter spp. (CPY; 109)	>2	>2	32	32	8
Salmonella spp. (SAL; 101)	0.03	0.12	16	>256	≤0.5
Shigella spp. (SHI; 101)	0.008	0.016	16	>256	>64
Yersinia spp. (YER; 46)	0.016	0.03	8	32	2
Vibrio spp. (VIB; 20)	0.03	0.06	1	8	≤0.5

ULI was highly active against all isolates and species tested (MIC_{50}, ≤0.03 µg/ml; MIC_{90}, ≤0.06 µg/ml) except for CPY (MIC_{50}, 0.03 µg/ml; MIC_{90}, >2 µg/ml). CIP displayed a similar spectrum of activity to ULI against all species tested, but exhibited 2- to 4-fold less activity (MIC_{90} range, 0.016 to 0.12 versus 0.008 to 0.03 µg/ml, respectively). Elevated ULI MIC results (≥4 µg/ml) were seen for rare AER, CPY, and EC; conversely, all SAL, SHI, YER, and VIB were inhibited by ≤0.12 µg/ml. ULI was highly active against all targeted subgroups of EC, and was 2-fold more active than CIP (MIC_{90}, 0.008 versus 0.016 µg/ml); only 3% of strains would be considered resistant using current CLSI ciprofloxacin breakpoints (one EHEC and two bloodstream EC).

Conclusions: Presently approved for clinical use in certain European countries and Japan, ULI was the most potent of the antimicrobial agents tested against TD pathogens in this study.

INTRODUCTION

The most common bacterial pathogens associated with gastroenteritis include *E. coli*, *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Vibrio* spp., *Campylobacter* spp., *Aeromonas* spp. and *Pleisiomonas shigelloides*. Although the most common manifestation of gastrointestinal infection is diarrhea, serious and invasive infections related to these bacterial species can occur, particularly among the young, elderly and immunocompromised patient populations.

The presentation of illness and the overall outcome can differ depending upon the causative agent. For example, isolates of *E. coli* associated with gastroenteritis can cause diverse symptoms and disease processes depending upon the toxins produced or the invasive characteristics of the offending organism. Enterotoxigenic, enteropathogenic, enteroaggregative and shiga toxin-producing (enterohemorrhagic) strains of *E. coli* can cause uncomplicated diarrheagenic symptoms (watery stool) or more serious problems such as hemolytic-uremic syndrome, malnutrition and life-threatening extra-intestinal infection. *Campylobacter* spp., *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. are well known pathogens associated with bacillary dysentery causing significant morbidity and mortality worldwide. *Y. enterocolitica*, *P. shigelloides*, *Aeromonas* spp. are less common causes of gastroenteritis but can also cause serious invasive disease.

Ulifloxacin (AF3013; NM394) is the resulting active metabolite of prulifloxacin following oral administration and intestinal absorption. This fluoroquinolone has potent in vitro activity against Gram-negative pathogens and maintains high concentrations within the gastrointestinal tract. The design of this study was to profile and assess the activity of ulifloxacin and other orally administered antimicrobial agents tested against a global collection of the eight major pathogen groups that are among known causes of bacterial gastroenteritis and traveler's diarrhea.

MATERIALS AND METHODS

Isolates were collected from medical centers worldwide (total; North America/Europe/Latin America) as follows: *Aeromonas* spp. (101; 38/45/18), *Campylobacter* spp. (109; 45/64/0), *Salmonella* spp. (101; 46/41/14), *Shigella* spp. (101; 35/33/33), *Yersinia* spp. (46; 6/39/1), *Escherichia coli* (100; 40/34/16), *Vibrio* spp. (20; 18/2/0), *P. shigelloides* (four; all NA). Footnotes for Table 1 and Table 2 list the number of tested species within each of the listed pathogen groups.

Isolates were tested in cation-adjusted Mueller-Hinton broth (CAMHB) using broth microdilution reference methods recommended by the Clinical and Laboratory Standards Institute (CLSI; M7-A7 [2006]). *Campylobacter* spp. were also tested using broth microdilution in CAMHB supplemented with 3-5% lysed horse blood using inoculum and incubation recommendations by the CLSI M45-A (2006). Interpretive criteria for susceptibility were those of the CLSI (M100-S17 [2007] and M45-A [2006]). Ulifloxacin breakpoint criteria are those suggested by Montanari (2005). Quality control (QC) strains utilized *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. jejuni* ATCC 33560. All QC results for the tested agents (ciprofloxacin, nalidixic acid, ampicillin, erythromycin, doxycycline and trimethoprim/sulfamethoxazole) were found to be in range (CLSI; M100-S17 [2007] or M45-A [2006]).

RESULTS

- Ulifloxacin was highly active against all tested Enterobacteriaceae isolates (MIC_{50}, ≤0.008 µg/ml; MIC_{90}, ≤0.03 µg/ml; see Table 1). While elevated ulifloxacin MIC values (≥2 µg/ml) were observed for rare strains of *E. coli*, 97.0% of *E. coli* isolates and all *Salmonella* spp., *Shigella* spp. and *Yersinia* spp. were susceptible to ulifloxacin.
- Ciprofloxacin and ulifloxacin displayed similar spectrums of activity against all species tested, but ciprofloxacin generally remained two- to four-fold less potent (MIC_{90} values, 0.016 to 0.12 µg/ml versus 0.008 to 0.03 µg/ml, respectively).
- Among *Aeromonas* spp., *P. shigelloides* and *Vibrio* spp., susceptibility to ulifloxacin was ≥98.0%, with slightly lower potency (MIC_{90}, 0.03-0.06 µg/ml) compared to the enteric bacilli (Table 2).

Table 1. In vitro activity of ulifloxacin and comparator antimicrobial agents tested against 348 isolates of Enterobacteriaceae.

Bacterial species (no.)/ Antimicrobial agent	MIC (µg/ml)			% category[a]	
	50%	90%	Range	Susceptible	Resistant
E. coli (100)[b]					
Ulifloxacin	0.008	0.008	0.004->2	97.0	3.0
Ciprofloxacin	0.008	0.016	0.004->2	97.0	3.0
Nalidixic acid	≤8	≤8	≤8->32	95.0	5.0
Rifaximin	8	16	4-32	-[c]	-
Ampicillin	2	>256	1->256	63.0	37.0
Doxycycline	2	16	0.25-32	76.0	16.0
Trimethoprim/sulfamethoxazole	≤0.5	>64	≤0.5->64	75.0	25.0
Salmonella spp. (101)[d]					
Ulifloxacin	0.008	0.03	0.002-0.12	100.0	0.0
Ciprofloxacin	0.008	0.12	0.004-0.25	100.0	0.0
Nalidixic acid	≤8	>32	≤8->32	84.2	15.8
Rifaximin	8	16	2->64	-	-
Ampicillin	1	>256	≤0.12->256	77.2	22.8
Doxycycline	2	32	0.5-64	82.2	13.9
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->64	91.1	8.9
Shigella spp. (101)[e]					
Ulifloxacin	0.004	0.008	0.002-0.12	100.0	0.0
Ciprofloxacin	0.008	0.016	0.004-0.25	100.0	0.0
Nalidixic acid	≤8	≤8	≤8->32	97.0	3.0
Rifaximin	8	16	2-16	-	-
Ampicillin	128	>256	0.25->256	40.6	59.4
Doxycycline	16	32	0.25-64	34.7	52.5
Trimethoprim/sulfamethoxazole	>64	>64	≤0.5->64	32.7	67.3
Yersinia spp. (46)[f]					
Ulifloxacin	0.008	0.016	0.008-0.12	100.0	0.0
Ciprofloxacin	0.016	0.03	0.008-0.25	100.0	0.0
Nalidixic acid	≤8	≤8	≤8->32	93.5	6.5
Rifaximin	4	8	1-16	-	-
Ampicillin	32	32	≤0.12-128	6.5	91.3
Doxycycline	1	2	≤0.12-16	95.7	4.3
Trimethoprim/sulfamethoxazole	≤0.5	2	≤0.5-8	93.5	6.5

a. Percent susceptible and resistant were based on CLSI criteria (M100-S17; 2007). Ulifloxacin susceptibility was based on 1 µg/ml = susceptible, 2 µg/ml = intermediate and 4 µg/ml = resistant [Montanari et al., 2005].
b. Included: Enterohemorrhagic *E. coli* (EHEC; 21 strains), enteropathogenic *E. coli* (EPEC; 20 strains), enteroaggregative *E. coli* (EAEC; 19 strains), enterotoxigenic *E. coli* (ETEC; 20 strains) and 20 isolates of invasive (bloodstream) isolates of *E. coli*.
c. - = no established breakpoint criteria.
d. Included: *S. typhimurium* (30 strains), *S. enteritidis* (30 strains), *S. typhi* (20 strains), *S. paratyphi* (10 strains), *S. choleraesuis* (four strains), *S. newport* (three strains), *S. virchow* (three strains) and *S. heidelberg* (one strain).
e. Included: *S. sonnei* (45 strains), *S. flexneri* (26 strains), *S. boydii* (18 strains) and *S. dysenteriae* (12 strains).
f. Included: *Y. enterocolitica* (44 strains) and *Y. pseudotuberculosis* (two strains).

- Rifaximin was most active against *Vibrio* spp. isolates (MIC_{50}, 0.5 µg/ml) but less so against *Aeromonas* spp., *P. shigelloides* (4 to 8 µg/ml) and *Campylobacter* spp. (32 µg/ml); against subgroups of *E. coli*, ulifloxacin was 2048-fold more active than rifaximin (MIC_{90}, 0.008 versus 16 µg/ml).
- Doxycycline was most active against *Yersinia* spp., *Aeromonas* spp., *P. shigelloides* and *Vibrio* spp. (>95% susceptible) and least active against other Enterobacteriaceae (34.7 to 82.2%).
- While fluoroquinolone and doxycycline resistance were detected among *Campylobacter* spp. (14.7 to 16.5% and 33.9%, respectively), all isolates were susceptible to erythromycin.

Table 2. In vitro activity of ulifloxacin and comparator antimicrobial agents tested against 234 strains of other enteric gram-negative pathogens.

Bacterial species (no.)/ Antimicrobial agent	MIC (µg/ml)			% category[a]	
	50%	90%	Range	Susceptible	Resistant
Aeromonas spp. (101)[b]					
Ulifloxacin	0.004	0.06	0.002->2	98.0	2.0
Ciprofloxacin	0.004	0.06	≤0.001->2	98.0	2.0
Nalidixic acid	≤8	>32	≤8->32	81.2	18.8
Rifaximin	4	8	0.5-16	-[c]	-
Ampicillin	>256	>256	0.25->256	1.0	98.0
Doxycycline	0.5	1	≤0.12-4	100.0	0.0
Trimethoprim/sulfamethoxazole	≤0.5	8	≤0.5->64	85.1	14.9
Pleisiomonas shigelloides (4)					
Ulifloxacin	0.004	-	0.004-0.008	100.0	0.0
Ciprofloxacin	0.004	-	0.002-0.008	100.0	0.0
Nalidixic acid	≤8	-	≤8	100.0	0.0
Rifaximin	8	-	8	-	-
Ampicillin	64	-	64-128	0.0	100.0
Doxycycline	≤0.12	-	≤0.12-0.25	100.0	0.0
Trimethoprim/sulfamethoxazole	≤0.5	-	≤0.5-1	100.0	0.0
Vibrio spp. (20)[d]					
Ulifloxacin	0.016	0.03	0.002-0.03	100.0	0.0
Ciprofloxacin	0.016	0.06	≤0.001-0.06	-	-
Nalidixic acid	≤8	≤8	≤8-16	-	-
Rifaximin	0.5	1	0.25-8	-	-
Ampicillin	2	8	0.25-16	90.0	0.0
Doxycycline	≤0.12	≤0.12	≤0.12-0.25	100.0	0.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5-1	100.0	0.0
Campylobacter spp. (109)[e]					
Ulifloxacin	0.03	>2	0.008->2	84.4	14.7
Ciprofloxacin	0.06	>2	0.008->2	82.6	16.5
Nalidixic acid	≤8	>32	≤8->32	-	-
Rifaximin	32	32	16-64	-	-
Ampicillin	2	32	0.25-128	-	-
Erythromycin	0.25	0.5	≤0.06-2	100.0	0.0
Doxycycline	≤0.12	16	≤0.12-32	59.6	33.9
Trimethoprim/sulfamethoxazole	2	8	≤0.5-32	-	-

a. Percent susceptible and resistant were based on CLSI criteria (M100-S17 [2007] and M45-A [2006]). Ulifloxacin susceptibility was based on 1 µg/ml = susceptible, 2 µg/ml = intermediate and 4 µg/ml = resistant [Montanari et al., 2005].
b. Included: *A. hydrophila* (86 strains), *A. caviae* (six strains), *A. sobria* (six strains) and *A. veronii* (three strains).
c. - = no established breakpoint criteria.
d. Included: *V. cholerae* (five strains), *V. parahaemolyticus* (eight strains), *V. alginolyticus* (two strains), *V. fluvialis* (two strains), *V. vulnificus* (two strains) and *Vibrio* spp. (one strain).
e. Included: *C. jejuni* (104 strains) and *C. coli* (five strains).

CONCLUSIONS

- Ulifloxacin was the most active of all orally administered antimicrobial agents tested, and exhibited a spectrum of activity against this global collection of gastroenteritis pathogens most similar to that of ciprofloxacin, but was two- to four-fold more potent than that agent.
- Rare, ulifloxacin-resistant strains of *Aeromonas* spp. and *E. coli* were identified, however the MIC_{90} values for these species were acceptably low at 0.06 and 0.008 µg/ml, respectively; *Campylobacter* spp. displayed a higher resistance rate to ulifloxacin (14.7% of isolates at ≥2 µg/ml), similar to that observed with ciprofloxacin.
- Isolates of *E. coli, Aeromonas* spp., *Salmonella* spp., *Shigella* spp., and *Yersinia* spp. displaying resistance to nalidixic acid and susceptibility to ciprofloxacin is a worrisome finding, being indicative of first-step mutations in the quinolone-resistance-determining region (QRDR).
- Ulifloxacin, the active metabolite of prulifloxacin, displays significant activity against the most commonly isolated gastroenteritis-producing bacterial pathogens, warranting further consideration for this clinical indication.

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