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Effects of Varying MIC parameters on the *in vitro* Activity of Finafloxacin

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Revised Abstract

Background: Finafloxacin is a novel member of the fluoroquinolone class of antibiotics. Specifically, finafloxacin belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions, where the activity of many existing fluoroquinolones is impaired. **Methods:** This CLSI M23 Tier 1 study investigated the effects of thirteen different MIC parameters: atmosphere, bovine serum albumin, calcium, fresh versus frozen plates, incubation time, inoculum size, medium, magnesium, plasma, pH, polysorbate 80, serum and temperature. The effect of varying each parameter on MICs was determined according to CLSI M07-A8 microdilution methodology. **Results:** Of the 13 different parameters studied, only pH consistently affected activity against all species, addition of plasma or serum was more species specific (see Table): All other parameters had no effect on the activity.

Species (n)	Standard Controls*	pH 5.8	pH 6.5	pH 8.5	50% plasma	25% serum	50% serum
H. influenzae (30)	0.008	0.03	0.04	0.36	NG**	0.009	0.006
K. pneumoniae (30)	2.2	0.644	0.401	6.8	10.037	3.81	1.54
P. aeruginosa (30)	12.1	4.6	6.1	24.8	5.104	10.752	13.803
S. aureus (30)	0.75	0.55	0.27	7.1	5.879	2.175	4.467
S. pneumoniae (30)	5.7	0.015	1.883	9.4	0.019	5.9	8.333
S. pyogenes (30)	0.558	0.015	0.224	2.4	0.015	0.625	1.4

Values shown are mean MICs expressed in mcg/ml. *Standard control conditions run at pH 7.2 with no added plasma or serum; **NG, no growth. Shading represents statistically significant differences (p<0.05) as compared to standard conditions.

Conclusions: Compared with the activity of finafloxacin under standard CLSI M07-08 conditions, pH affected the activity of the drug against all species studied. At lower pH, finafloxacin was more active while at higher pH the drug was less active. Plasma and serum also affected finafloxacin activity though the effects were more species specific.

Introduction

Finafloxacin is a novel member of the fluoroquinolone class of antibiotics. Specifically, finafloxacin belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions, where the activity of many existing fluoroquinolones is impaired. Finafloxacin is currently in clinical development with MerLion Pharmaceuticals. Under license with Alcon Pharmaceuticals, USA, finafloxacin is being developed for treating ear infections, including acute otitis externa (outer ear and ear canal) and acute otitis media (middle ear). In addition to otic and ophthalmic indications, finafloxacin, which has already shown potential for the treatment of a range of infections in Phase IIa clinical studies, is also being evaluated in critical care and hospital based infection settings

In order to thoroughly understand the activity of finafloxacin, an M23-A3 Tier 1 study in line with the requirements set out by the Clinical and Laboratory Standards Institute (CLSI) was performed. Quality control testing in the present study includes investigating the effects of thirteen different MIC parameters on the activity of finafloxacin against standard CLSI QC organisms and clinical isolates, namely, medium, fresh versus frozen panels, incubation time, calcium, magnesium, polysorbate 80, serum, plasma, pH, inoculum size, temperature, atmosphere and bovine serum albumin.

Materials & Methods

Parameter Modifications

Test media composition (finafloxacin and moxifloxacin): Modifications to the test media composition were made to result in the following systematic changes: 25 mcg/ml Ca^{++}, 50 mcg/ml Ca^{++}, 100 mcg/ml Ca^{++}, 12.5 mcg/ml Mg, 25 mcg/ml Mg, pH 5.8, pH 6.5, pH 7.2, pH 8.5, 25% human serum, 50% human serum, 50% human plasma, 0.002% polysorbate 80, and supplemented with bovine serum albumin at 0.001%, 0.05%, 0.02%, and 0.1%.

Incubation time and conditions (finafloxacin and moxifloxacin): The effects of incubation conditions were evaluated by incubating plates in ambient air, 5% CO_2 and under anaerobic conditions. Standard test panels were incubated at 30º C, 35º C and 40º C. Variations to inoculum preparation were evaluated by testing concentrations of 10^4, 10^5, 10^6, and 10^7 CFU/ml. All concentrations were confirmed by colony count. Standard test panels were read at 16, 18, 20, 24, and 48 hours to evaluate incubation time.

Panels (finafloxacin and moxifloxacin): Standard panels frozen for 48 hours at -80ºC were set up under standard testing conditions.

Antimicrobial Susceptibility Testing

Minimum inhibitory concentration (MIC) endpoints were determined by broth microdilution according to CLSI guidelines (CLSI, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard- Eighth Edition*, M07-A8, 2009). All panels were produced at IHMA.

Cation-adjusted Mueller-Hinton broth (CAMHB), cation-adjusted Mueller-Hinton broth supplemented with 3% laked horse blood (MHB/LHB) and *Haemophilus* Test Medium broth (HTM) were used as the test media. Endpoints were determined following CLSI guidelines (CLSI, *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement* M100-S21, 2011).

Results

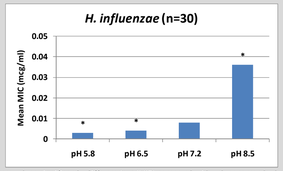
Of the thirteen different MIC parameters tested, only pH and to a lesser extent plasma and serum affected the activity of finafloxacin. Atmospheric conditions, calcium, magnesium, incubation time, inoculums size, medium, polysorbate 80, temperature and the use of fresh or frozen susceptibility plates had no effect on the activity of finafloxacin. Consequently, only data for pH, serum and plasma are shown below.

Table 1. Effect of pH on finafloxacin and moxifloxacin MIC activity (mcg/ml) against ATCC Quality Control Strains.

	Finafloxacin MICs (mcg/ml)			
Organism	**pH 5.8**	**pH 6.5**	**pH 7.2**	**pH 8.5**
E. coli ATCC 25922	≤.015	≤.015	.03	.25
E. faecalis ATCC 29212	.5	.25	.5	2
H. influenzae ATCC 49247	NG	≤.004	.008	NG
P. aeruginosa ATCC 27853	.25	2	4	32
S. aureus ATCC 29213	.12	.03	.12	1
S. pneumoniae ATCC 49619	≤.015	.25	.5	2
	Moxifloxacin MICs (mcg/ml)			
	pH 5.8	**pH 6.5**	**pH 7.2**	**pH 8.5**
E. coli ATCC 25922	1	.06	.03	≤.015
E. faecalis ATCC 29212	2	.25	.25	.12
H. influenzae ATCC 49247	NG	.03	.015	NG
P. aeruginosa ATCC 27853	32	16	4	2
S. aureus ATCC 29213	1	.12	.06	.06
S. pneumoniae ATCC 49619	≤.015	.12	.12	.06

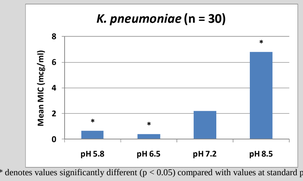
NG, no growth.

Figure 1. Effect of pH on activity of finafloxacin against clinical isolates of *H. influenzae*.



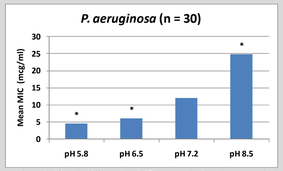
* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 2. Effect of pH on activity of finafloxacin against clinical isolates of *K. pneumoniae*.



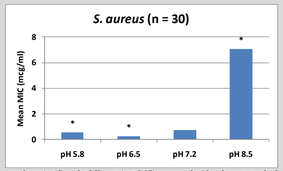
* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 3. Effect of pH on activity of finafloxacin against clinical isolates of *P. aeruginosa*.



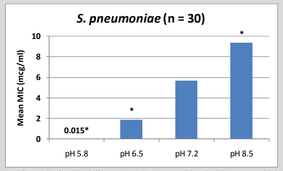
* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 4. Effect of pH on activity of finafloxacin against clinical isolates of *S. aureus*.



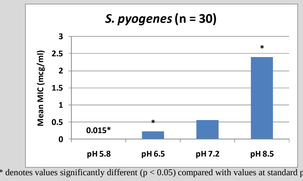
* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 5. Effect of pH on activity of finafloxacin against clinical isolates of *S. pneumoniae*.



* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Figure 6. Effect of pH on activity of finafloxacin against clinical isolates of *S. pyogenes*.



* denotes values significantly different (p < 0.05) compared with values at standard pH 7.2

Table 2. Effect of serum / plasma on finafloxacin and moxifloxacin MIC values against ATCC Quality Control Strains.

	Finafloxacin MICs (mcg/ml)			
Organism	**Control**	**25% Serum**	**50% Serum**	**50% Plasma**
E. coli ATCC 25922	.03	.12	.06	≤.015
E. faecalis ATCC 29212	.5	1	2	≤.015
H. influenzae ATCC 49247	.008	.008	NG	NG
P. aeruginosa ATCC 27853	8	8	4	2
S. aureus ATCC 29213	.12	.25	.5	≤.015
S. pneumoniae ATCC 49619	.5	1	2	≤.015
	Moxifloxacin MICs (mcg/ml)			
	Control	**25% Serum**	**50% Serum**	**50% Plasma**
E. coli ATCC 25922	.03	≤.015	≤.015	≤.015
E. faecalis ATCC 29212	.12	.12	.06	≤.015
H. influenzae ATCC 49247	.008	.008	NG	NG
P. aeruginosa ATCC 27853	4	1	.25	.03
S. aureus ATCC 29213	.03	.03	.03	≤.015
S. pneumoniae ATCC 49619	.12	.12	.12	≤.015

NG, no growth.

Table 3. Effect of serum / plasma on finafloxacin and moxifloxacin activity against clinical isolates.

			Finafloxacin MICs (mcg/ml)	
	Control	50% Plasma	25% Serum	50% Serum
H. influenzae	0.009	NG	0.009	0.006
K. pneumoniae	2.286	10.037	3.818	1.542
P. aeruginosa	13.067	5.104	10.752	13.803
S. aureus	1.355	5.879	2.175	4.467
S. pneumoniae	3.95	0.019	5.9	8.333
S. pyogenes	0.317	≤0.015	0.625	1.4

			Moxifloxacin MICs (mcg/ml)	
	Control	50% Plasma	25% Serum	50% Serum
H. influenzae	0.01	NG	0.008	0.005
K. pneumoniae	1.192	0.214	0.29	0.066
P. aeruginosa	10.967	0.069	2.33	1.899
S. aureus	2.43	3.346	2.021	2.421
S. pneumoniae	0.944	0.019	0.731	0.787
S. pyogenes	0.142	≤0.015	0.185	0.172

*Values represent arithmetic means. NG, no growth. Grey shading represents values that were statistically significantly different (p < 0.05) as compared with control values.

Conclusions

- Of the thirteen parameters studied, differences in pH most commonly affected the activity of finafloxacin.
- The activity of finafloxacin at low pH (pH 5.8 and 6.5) was generally superior (p < 0.05) against all isolates tested when compared with control pH (7.2).
- The addition of plasma caused an increase in activity against three species and a decrease in activity for two species.
- The addition of 25% and 50% serum caused a decrease in finafloxacin activity against *S. aureus*, *S. pneumoniae* and *S. pyogenes*. However, there was no effect on finafloxacin activity against *K. pneumoniae* and *P. aeruginosa*.
- All other parameters including atmospheric conditions, calcium, magnesium, incubation time, inoculums size, medium, polysorbate 80, temperature and the use of fresh or frozen susceptibility panels had no effect on the activity of finafloxacin.