Antibacterial Activity of Finafloxacin Against Isogenic *Pseudomonas aeruginosa* (*Pa*) Isolates Expressing Combinations of Defined Mechanisms of Fluoroquinolone (FQ) Resistance and Propensity to Select for Resistance.

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Abstract

Introduction
Finafloxacin (FIN) is a novel fluoroquinolone (FQ) that exhibits improved antibacterial and pharmacodynamic properties at pH values below neutral which often characterize infection sites. The aim of this study was to determine the propensity of FIN and other FQs to select for resistance in the nosocomial pathogen *Pa* and to determine the effects of defined target and efflux resistance mechanisms on activity.

Methods
Mutation prevention concentrations (MPC) for FIN, ciprofloxacin (CIP), levofloxacin (LVX) were determined for *Pa* 27853 from an inoculum of 10^{10} CFU/mL at pH 7.2, 6.2 and 5.2. Susceptibility testing was also performed at these pHs (CLSI method).

Results
MPCs for FIN were 64, 8 and 8 mg/L at pH 7.2, 6.2 and 5.2 respectively, for CIP (2, 8 and 64) and for LVX (8, 32 and 64). MPC/MIC ratios were in the range 4-16, resistance frequencies were in the range 3.4 x10^{-8} - 1.9 x10^{-9}, and mutant MICs were 4-16-fold higher than that of the parent. The susceptibility of isogenic sets of *Pa* harboring defined mutations in efflux regulators, target mutations and combinations thereof are shown in the Table.

Conclusions
Unlike marketed FQs, FIN activity increases at low pH. This was reflected in the greater potency of FIN towards FQ resistant *Pa* and having a lower propensity, than CIP or LVX, to select for resistant *Pa* at the more acidic pH. These data suggest that FIN could have an advantage over other FQs at sites acidified by infection and inflammation processes.

Background

- Finafloxacin is a novel pH activated, broad spectrum fluoroquinolone in development for infection indications in the hospital and critical care setting [1]
- Finafloxacin exhibits enhanced activity at low pH and under other environmental conditions associated with infection [1, 2]
- Finafloxacin exhibits bactericidal activity against forms of quiescent growth, thought to be relevant *in vivo* e.g. non-growing cells, biofilms and persisters [3]
- Other fluoroquinolones lose activity under such conditions. Consequently, finafloxacin exhibited superior activity in a series of infection models [4,5]
- The activity of finafloxacin under infection relevant conditions and against infection relevant growth forms in combination with the high dosing potential predicted from its safety profile [6, 7, 8], suggest finafloxacin will offer improved properties over currently marketed fluoroquinolones.

References

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Background and aim

Pseudomonas aeruginosa is an important hospital pathogen which is becoming increasingly difficult to treat due to the emergence and selection of resistant strains. Resistance to fluoroquinolones is usually mediated through combinations of target mutations in *gyrA* and mutations giving rise to increased activity of efflux pumps.

The aims of this study were to investigate the propensities of finafloxacin and other marketed fluoroquinolones to select for resistance in *P. aeruginosa* and to measure their activities against strains harboring combinations of mutations within target and regulatory genes affecting drug efflux.

Methods

- MICs were determined in pH adjusted cation adjusted Mueller-Hinton broth (MHB) using CLSI methodology for broth microdilution.
- The following antibiotics (with abbreviations) were tested: ciprofloxacin (CIP), finafloxacin (FIN) and levofloxacin (LVX). The putative efflux pump inhibitor (EPI) phenylalanyl-arginyl-ß-naphthylamide (PAßN) was added to 8 µg/mL to examine the role of efflux in wild type and MDR backgrounds.
- *P. aeruginosa* strain ATCC 27853 was used for determination of mutation prevention concentration (MPC) and mutation frequency. Inocula of 10^{10} colony forming units were spread onto a series of Mueller-Hinton agar (MHA) plates containing 2-fold dilutions of the test drug. The lowest concentration at which no mutants grew following 48 h incubation was the MPC.
- To examine the effects of target mutations, regulatory mutations affecting multiple drug efflux (MDE) pumps and combinations thereof, *P. aeruginosa* strain ML5087 was used. Mutants containing MDE resistance markers in: *nfxB* (overexpression of MexCD-OprJ), *nalB* / *mexR* (overexpression of MexAB-OprM) and *nfxC* (overexpression of MexEF-OprN) were constructed with and without further quinolone resistance mutations within the target gene *gryA*. Mutations were confirmed by sequencing, details of which are listed in Table 2.

Results

	pH 5.2			pH 6.2			pH 7.2		
	FIN	CIP	LVX	FIN	CIP	LVX	FIN	CIP	LVX
MIC [mg/L]	1	4	4	1	1	2	4	0.25	1
MPC [mg/L]	8	64	64	8	8	32	64	2	8
MPC/MIC	8	16	16	8	8	16	16	8	8
Mutation frequency (at 1/2 MPC)	8.2 x 10^{-9}	2.7 x 10^{-8}	4.3 x 10^{-8}	2.0 x 10^{-8}	3.4 x 10^{-8}	7.4 x 10^{-9}	1.6 x 10^{-8}	1.8 x 10^{-9}	5.1 x 10^{-9}

Table 1: Mutation prevention concentrations and mutation frequencies of finafloxacin, ciprofloxacin and levofloxacin, determined at pH 5.2 pH 6.2 and pH 7.2 determined with *Pa* ATCC 27853

- The MPC of FIN decreased at acidic pH compared to pH 7.2, conversely the MPCs of CIP and LVX increased. Mutants to FIN, CIP and LVX exhibited proportional 4-16-fold increases in MIC to the other FQs.

Strain	Fluoroquinolone resistance marker	Upregulated efflux pump	MIC [mg/L]pH 5.2			MIC [mg/L]pH 6.2			MIC [mg/L]pH 7.2		
			FIN	CIP	LVX	FIN	CIP	LVX	FIN	CIP	LVX
ML5087	Wild type		0.5	1	4	0.5	0.25	0.5	8	0.125	0.5
ML5087-M1a	*gyrA*T83I		16	16	64	16	16	32	>64	8	32
ML5087	nfxBD11bp	MexCD-OprJ	0.5	4	8	0.5	0.125	0.5	4	0.125	0.5
ML5087nfxB-M13a	nfxBD11bp, *gyrA*T83I	MexCD-OprJ	8	64	>64	16	4	16	>64	8	16
ML5087	nalB (=*mexR*T130P)	MexAB-OprM	2	16	32	4	0.5	2	64	0.5	4
ML5087nalB-M6a	nalB (=*mexR*T130P), *gyrA*T83I	MexAB-OprM	16	>64	>64	32	32	64	>64	16	32
ML5087-M4	nfxC	MexEF-OprN	2	16	32	4	2	8	32	0.5	2
ML5087nfxC-M4-M8	nfxC, *gyrA* T83I	MexEF-OprN	16	64	>64	32	32	64	>64	16	32

Table 2: MICs of finafloxacin, ciprofloxacin and levofloxacin, at pH 5.2 pH 6.2 and pH 7.2, against isogenic *P. aeruginosa* harboring combinations of target and MDE fluoroquinolone resistance mutations.

- Mutations in nalB and nfxC, resulting in increased expression of MexAB-OprM and MexEF-oprN respectively, resulted in MIC increases for all FQs by 4 to 32-fold.
- Combination of a *gyrA* and MDE mutation resulted in MIC increases of 16 to >128-fold, relative to wild type.
- Lowering pH from 7.2 to 6.2 and 5.2 increased the activity of finafloxacin by a factor of 4 to 32-fold. Conversely, the activities of ciprofloxacin and levofloxacin decreased at the lower pHs by a factor of up to 32-fold.

Strain	FQ resistance marker	pH 5.2						pH 6.2						pH 7.2					
		FIN		CIP		LVX		FIN		CIP		LVX		FIN		CIP		LVX	
			+EPI		+EPI		+EPI		+EPI		+EPI		+EPI		+EPI		+EPI		+EPI
Wild type		0.5	0.125	1	0.5	4	1	0.5	0.06	0.25	≤0.06	0.5	≤0.06	8	1	0.125	≤0.06	0.5	≤0.06
nfxBD11bp	mexCD-oprJ	0.5	0.125	4	1	8	2	0.5	0.125	0.125	0.125	0.5	0.125	8	2	0.125	≤0.06	0.5	0.125
nalB (=*mexR*T130P)	mexAB-oprM	2	0.25	16	4	32	8	4	0.5	0.5	0.25	2	0.5	64	16	0.5	0.25	4	1
nfxC	mexEF-oprN	2	1	32	16	32	16	4	1	2	2	4	1	32	8	0.5	0.5	2	1

Table 3: MICs of isogenic strains harboring MDR mutations, in the presence and absence of efflux pump inhibitor (EPI)

- Addition of the putative efflux pump inhibitor PAßN partially restored sensitivity in both wild type and MDE backgrounds, suggesting that efflux plays a role both in the intrinsic resistance of the strain and in the reduced susceptibility of the MDE mutants.

CIP; ciprofloxacin, EPI; efflux pump inhibitor (PAßN at 8 mg/L) FIN; finafloxacin, LVX; levofloxacin, MPC; mutation prevention concentration

Conclusions

- **Finafloxacin activity against *P. aeruginosa* was reduced by the presence of mutations in the target gene (*gyrA*) and MDE backgrounds *nfxB*, *nfxC* and *nalB*, to a similar degree to other fluoroquinolones. Despite this, the pH activation exhibited by finafloxacin resulted in an overall greater potency than ciprofloxacin and levofloxacin against fluoroquinolone resistant *P. aeruginosa* under acidic conditions (Table 2).**
- **The pH activation also translated into finafloxacin exhibiting a lower potential than ciprofloxacin or levofloxacin to select for resistance under acidic conditions (Table 1).**
- **Based on these findings, finafloxacin could exhibit improved antibacterial properties over other fluoroquinolones, against *P. aeruginosa* in infection sites acidified by inflammation and other physiological processes relating to infection.**