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Activity of the Investigational Fluoroquinolone Finafloxacin Against Ciprofloxacin-Sensitive and -Resistant *Acinetobacter baumannii*

C1-094

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Abstract

Background: *Acinetobacter baumannii* is a serious nosocomial pathogen characterised by its innate and acquired resistance to most antimicrobials, including fluoroquinolones (FQ). FQ resistance is mediated through target site mutations in *gyrA* and *parC* combined with increased efflux. Finafloxacin (FIN) is a novel fluoroquinolone which shows enhanced activity under acidic pH where all other FQ lose activity. This study investigated the activity of FIN and ciprofloxacin (CIP) against *A. baumannii* isolates with characterised resistance mechanisms.

Methods: 72 *A. baumannii* clinical isolates were included. CIP and FIN MICs were performed by agar dilution under standard conditions (pH 7.2) or at a pH of 5.8.

Results: Results are summarised in Table 1. At pH 7.2 FIN had comparable activity to CIP. Activity at pH 5.8 showed a dramatic lowering in MIC with FIN. In contrast, CIP MICs rose under these conditions. FIN MICs are raised with a GyrA substitution but are less affected by an additional ParC substitution.

Conclusions: Overall, FIN demonstrated superior activity to CIP under acidic conditions against all isolates irrespective of their resistance mechanism. Furthermore, FIN showed comparable activity to CIP at pH 7.2. Hence, FIN could be a promising new antimicrobial agent for the treatment of *A. baumannii* infections at acidic body compartments.

Introduction and Purpose

- *Acinetobacter baumannii* is a serious nosocomial pathogen characterised by its innate and acquired resistance to most antimicrobials, including fluoroquinolones.
- Fluoroquinolones resistance is mediated through target site mutations in *gyrA* and *parC* combined with increased efflux.
- Finafloxacin (Figure 1) is a novel fluoroquinolone which shows enhanced activity under acidic pH where all other fluoroquinolones lose activity.
- This study investigated the activity of finafloxacin and ciprofloxacin against *A. baumannii* isolates with characterised resistance mechanisms.

Methods

- **Bacterial isolates:** A total of 72 *A. baumannii* clinical isolates were investigated. Of these, 69 have characterised *gyrA* and *parC* genes (1). Four strains overexpress the efflux pump *adeB* compared to their isogenic parent strains (2).
- **Sensitivity testing:** Ciprofloxacin and finafloxacin MICs were determined by agar dilution under standard conditions (pH 7.2) or at a pH of 5.8. Mueller Hinton agar was prepared following the manufacturers instructions and the pH was adjusted with HCl prior to pouring into petri dishes. pH of the agar was checked once solidified.

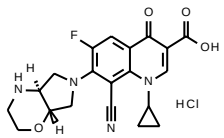
Results

Table 1. Agar dilution MICs of ciprofloxacin (CIP) and finafloxacin (FIN) under normal pH (7.2) and acidic (pH 5.8) conditions against characterised *A. baumannii* isolates.

	Amino acid substitutions		MIC (µg/ml)			
Strain No.	GyrA	ParC	CIP normal	FIN normal	CIP acidic	FIN acidic
1	-	-	0,06	0,12	2	0,06
2	-	-	0,06	0,5	2	0,12
3	-	-	0,12	0,12	2	0,06
4	-	-	0,12	0,25	1	0,03
5 - 7	-	-	0,12	0,25	2	0,06
8 - 10	-	-	0,25	0,25	2	0,12
11	-	-	0,25	0,25	4	0,06
12	-	-	0,5	1	2	0,12
13	-	-	0,5	1	4	0,12
14	-	-	0,5	1	4	0,25
15 - 17	-	-	1	1	4	0,12
18	-	-	1	1	8	0,12
19	Ser83-Leu	-	1	0,5	4	0,12
20	Ser83-Leu	-	2	1	4	0,06
21	Ser83-Leu	-	4	1	32	0,25
22	Glu87-Gly	-	4	8	32	1
23 - 25	Ser83-Leu	-	4	16	32	2
26, 27	Ser83-Leu	-	4	16	32	1
28	Ser83-Leu	-	4	32	32	4
29	Ser83-Leu	-	8	8	32	0,5
30	Ser83-Leu	-	8	16	32	1
31	Ser83-Leu	-	16	16	32	1
32	Ser83-Leu	-	8	16	128	1
33, 34	Ser83-Leu	-	8	16	128	2
35, 36	Ser83-Leu	-	16	16	128	2
37	Ser83-Leu	-	32	16	128	2
38-41	Ser83-Leu	-	16	16	>128	2
42	Ser83-Leu	-	32	16	>128	2
43	Ser83-Leu	Glu84-Lys	32	16	>128	4
44, 45	Ser83-Leu	Ser80-Leu	64	16	>128	2
46	Ser83-Leu	Ser80-Phe	64	32	>128	4
47, 48	Ser83-Leu	Ser80-Leu	128	16	>128	2
49	Ser83-Leu	Ser80-Leu	128	16	>128	4
50 - 52	Ser83-Leu	Ser80-Leu	128	32	>128	2
53 - 55	Ser83-Leu	Ser80-Leu	128	32	>128	4
56	Ser83-Leu	Ser80-Phe	128	32	>128	4
57	Ser83-Leu	Ser80-Leu	128	64	>128	4
58	Ser83-Leu	Glu84-Lys	128	64	>128	8
59 - 61	Ser83-Leu	Ser80-Leu	128	64	>128	8
62	Ser83-Leu	Glu84-Lys	>128	64	>128	2
63	Ser83-Leu	Ser80-Leu	>128	64	>128	4
64	Ser83-Leu	Ser80-Leu	>128	64	>128	8
65*	Ser83-Leu	-	32	16	128	2
66⁺	Ser83-Leu	Ser80-Leu	>128	64	>128	4
67⁺	Ser83-Leu	Ser80-Leu	>128	64	>128	4
68*	Ser83-Leu	-	4	8	32	1
69⁺	Ser83-Leu	-	128	128	>128	8
70*, 71*	nt	nt	128	32	>128	4
72⁺	nt	nt	>128	64	>128	4

*parent strain; ⁺*adeB* overexpressing; nt, not tested; strain 69 is a moxifloxacin-selected laboratory mutant derived from strain 68.

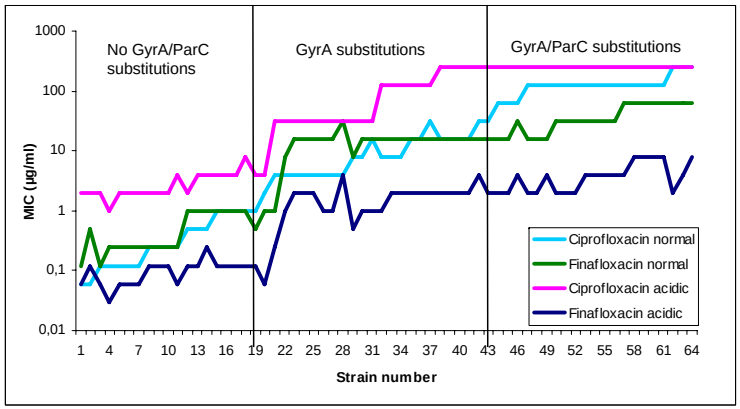
Figure 1. Structure of Finafloxacin HCl



Results cont.

- Results are summarised in Table 1 and shown graphically in Figure 1.
- At pH 7.2 finafloxacin had comparable activity to ciprofloxacin.
- Activity at pH 5.8 showed a dramatic lowering in MICs with finafloxacin. In contrast, ciprofloxacin MICs rose under these conditions (Table 1).
- Finafloxacin MICs are raised with a GyrA substitution but are less affected by an additional ParC substitution (Figure 2).

Figure 2. Chart comparing MICs of ciprofloxacin and finafloxacin under normal and acidic pH.



Conclusions

- Overall, FIN demonstrated superior activity to CIP under acidic conditions against all *A. baumannii* isolates irrespective of their resistance mechanism.
- Furthermore, FIN showed comparable activity to ciprofloxacin at pH 7.2.
- FIN could be a promising new antimicrobial agent for the treatment of *A. baumannii* infections at acidic body compartments.

References and Acknowledgements

1. Wisplinghoff et al. J. Antimicrob. Chemother. (2002). 51: 177-180
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Antibacterial Activity And Resistance Potential Of The Investigational Fluoroquinolone Finafloxacin and Moxifloxacin Against Clinical Isolates of Community Associated Methicillin Resistant *Staphylococcus aureus*

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Revised Abstract

Background:
The prevalence of CA-MRSA has increased significantly over the past 10 years and consequently the use of β-lactams for SSSI has been restricted, thus new treatment strategies are being sought. FIN is an investigational fluoroquinolone that exhibits enhanced antibacterial activity under acidic conditions which could make it an appropriate candidate for CA-MRSA cutaneous abscesses. The antibacterial activity and resistance potential of FIN and MXF were compared against clinical isolates of CA-MRSA.

Methods:
41 CA-MRSA isolates were obtained from patients in Singapore and the NARSA collection. MICs were determined using CLSI methodology for broth microdilution, with the pH adjusted to 7.2 or 5.8. Mutation prevention concentration (MPC) and resistance frequencies were determined for the strain USA300 on agar from an inoculum of 1 x 10^{10} CFU. DNA sequencing was carried out using conventional methods.

Results:
At pH 7.2 the activity (MIC_{50}, MIC_{90}) of FIN was (0.125, 2) which was similar to MXF (0.06, 2). At pH 5.8, FIN (0.06, 1) was more active than MXF (0.25, 8). Under acidic conditions, the MPCs of FIN and MXF were 0.5 and 2 mg/L and resistance frequencies (to 1/2 MPC) were 2.6 x 10^{-9} and 1.4 x 10^{-9} respectively against USA300. First-step mutants exhibited an 8-16-fold increase in MIC, attributed to S80F or E84K substitution within *grlA*.

Conclusions:
FIN is unusual among the FQs in that its activity is improved under acidic conditions as shown against these isolates of CA-MRSA for which the $MIC_{50,90}$ of FIN were 4-fold lower than MXF. The similar resistance frequencies and mutational target indicate a common mode of action, yet the 4-fold lower MPC suggest a lower propensity for FIN resistance selection under acidic conditions. These data suggest that FIN could be a promising FQ option for CA-MRSA therapy.

Introduction

- Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) which is currently undergoing phase II clinical assessment.
- Finafloxacin exhibits the unusual property of enhanced *in vitro* and *in vivo* activity at slightly acidic conditions (pH 5.0 – 6.0) under which other marketed FQs exhibit significantly reduced activity. This is also true for adherent and slowly growing bacteria.
- Finafloxacin may be advantageous for indications associated with a low pH environment and / or inflammation. A potential area of further clinical investigation is e.g. cSSSI including cutaneous abscesses caused by communuity associated MRSA (CA-MRSA). The activity of finafloxacin and comparators was measured at pH 7.2 and pH 5.8 against a panel of 41 CA-MRSA, recently isolated in Singapore.
- CA-MRSA clones generally remain susceptible to FQs, therefore potential antibiotics for this indication should exhibit a low propensity for resistance development. This was also investigated by determining mutation frequencies and mutation prevention concentrations at pH 7.2 and pH 5.8.

Methods

- **Strains** – 41 CA-MRSA isolates including major clones USA300, ST80-MRSA-IV (European clone), ST30-MRSA-IV (Oceanian clone) and ST59-MRSA-V (Taiwan clone) were collected from the National University Hospital Singapore, between 2003 – 2008 from patients with cutaneous abscess (n = 26), colonization (n = 3), wound infection (n = 2) paronychia (n = 1), foot infection (n = 1), bacteremia (n = 2), pneumonia (n = 1), osteomyelitis (n = 1), TKR infection (n = 1), endocarditis (n = 1), exfoliative dermatitis (n = 1) and conjunctivitis (n = 1). Seven (17%) out of 41 isolates were resistant to fluoroquinolones. USA-300 was obtained from NARSA.
- **Susceptibilty testing -** Minimum inhibitory concentrations (MICs) of finafloxacin, ciprofloxacin, moxifloxacin, linezolid, clindamycin, erythromycin and trimethoprim / sulfamethoxazole were determined by broth microdilution (CLSI).
- **Resistance selection -** Mutation prevention concentration (MPC) and resistance frequencies were determined on drug containing agar from an inoculum of 10^{10} CFU. Mutant stability was confirmed by MIC and *gyrA* and *grlA* sequenced.

Results and Discussion

- Susceptibilty data at pH 7.2 and pH 5.8 are shown in Table 1 and Figure 2. Finafloxacin was among the most potent compounds (basis MIC_{50}) at pH 7.2. The shift to pH 5.8 resulted in a 2-fold increase in the activity of finafloxacin whereas moxifloxacin (4-fold), ciprofloxacin (2-fold), clindamycin (8-fold), erythromycin (32-fold) and trimethoprim / sulfamethoxazole (2-fold) all exhibited decreased activity. Linezolid activity was not affected by pH.
- Finafloxacin was the most potent compound tested under slightly acidic conditions (pH 5.8), exhibiting MIC_{50} values that were 2-fold lower than trimethoprim / sulfamethoxazole, 4-fold lower than moxifloxacin, 16-fold lower than clindamycin or ciprofloxacin and 32-fold lower than linezolid.
- Mutation frequencies for the strain NRS-384 (USA-300) at ½ MPC were similar for all fluoroquinolones (Table 2). At pH 7.2, finafloxacin exhibited mutation prevention concentration that was 2- and 64-fold lower than moxifloxacin and ciprofloxacin, respectively. At pH 5.8, finafloxacin exhibited an MPC that was 4-fold lower than moxifloxacin and 128-fold lower than ciprofloxacin.
- Finafloxacin (moxifloxacin and ciprofloxacin) mutants exhibited E84K or S80I substitutions within *grlA* conferring an 8 - 16-fold reduction in susceptibility.

Results

Table 1. MIC_{50} and MIC_{90} of finafloxacin and comparator antibiotics against 41 CA-MRSA isolates at pH 7.2 and pH 5.8

	FIN	MXF	CLN	CIP	ERY	LZD	TMP/SMX
pH 5.8							
MIC_{50} [mg/L]	0.06	0.25	1	1	16	2	0.125 / 2.375
MIC_{90} [mg/L]	1	8	2	>32	>32	2	0.5 / 9.5
pH7.2							
MIC_{50} [mg/L]	0.125	0.06	0.125	0.5	0.5	2	0.06 / 1.19
MIC_{90} [mg/L]	2	2	0.25	8	>32	2	0.5 / 9.5

Abbreviations: CIP; ciprofloxacin, CLN; clindamycin, ERY; erythromycin, FIN; finafloxacin, LZD; linezolid, MXF; moxifloxacin, TMP/SMX; trimethoprim / sulfamethoxazole.

Figure 1. Finafloxacin HCl

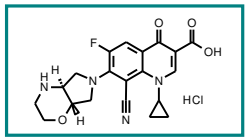
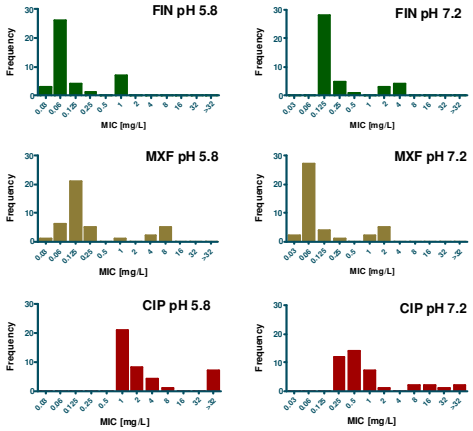


Table 2. Spontaneous resistance frequencies and mutation prevention concentration of finafloxacin, ciprofloxacin and moxifloxacin against *S. aureus* NRS-384 (USA-300) , selected at multiple drug concentrations at pH 7.2 and pH 5.8.

Drug conc. [mg/L]	Resistance frequency					
	pH 7.2			pH 5.8		
	MXF	CIP	FIN	MXF	CIP	FIN
0.125	CG		7.9×10^{-9}			1.4×10^{-8}
0.25	8.04×10^{-9}		2.6×10^{-9}	CG		2.6×10^{-9}
0.5	3.1×10^{-9}		**$< 1.2 \times 10^{-10}$**	9.6×10^{-9}		**$< 1.2 \times 10^{-10}$**
1	**$< 1.2 \times 10^{-10}$**			1.4×10^{-9}		
2		CG		**$< 1.2 \times 10^{-10}$**		
4		3.5×10^{-9}				
8		2.8×10^{-9}			CG	
16		2.2×10^{-9}			3.0×10^{-9}	
32		**$<1.9 \times 10^{-11}$**			2.3×10^{-9}	
64					**$<1.9 \times 10^{-11}$**	

Abbreviations: CG; Confluent growth, CIP; ciprofloxacin, FIN; finafloxacin, MXF; moxifloxacin. Mutation prevention concentration (MPC) shown in **bold.**

Figure 2. MIC distribution of finafloxacin, moxifloxacin and ciprofloxacin against 41 CA-MRSA isolates at pH 7.2 and pH 5.8



Conclusions

- **Finafloxacin exhibited superior activity to a panel of anti-staphylococcal antibiotics against CA-MRSA at pH 5.8.**
- **Finafloxacin also exhibited a lower propensity than moxifloxacin or ciproflxacin to select for spontaneous emergence of resistance at both pH 7.2 and pH 5.8.**
- **These properties of finafloxacin may be advantageous at infection sites with a pH range pH 5.0 – 7.0, e.g. cutaneous abscesses caused by CA-MRSA and warrants further clinical investigation.**

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Antibacterial activity of finafloxacin against isogenic *Escherichia coli* isolates expressing combinations of defined mechanisms of fluoroquinolone resistance

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Revised Abstract

Background:
Finafloxacin (FIN) is an investigational fluoroquinolone (FQ) which exhibits enhanced and broad spectrum activity under slightly acidic conditions (pH 5.0 - 7.2). The potency of FIN in comparison with ciprofloxacin (CIP), levofloxacin (LEV), and moxifloxacin (MOX) was investigated against mutants with known mechanisms of FQ resistance.

Methods:
MICs were determined at pH 5.8 and 7.2 for susceptible isolate *E. coli* WT and isogenic mutants which were FQ resistant due to either alteration in target genes (*gyrA*, *parC*), or inactivation of multiple drug resistance (MDR) efflux pump repressor (*marR*) and for isolates carrying transferable FQ resistance determinants; QnrA, B, and S, AAC(6')-Ib-cr, and QepA. Additionally, the impact of *qepA* on the FQ susceptibilities of WT and isogenic FQ resistant strains was determined.

Results:
The results are shown in Tables 1 and 2.
For all strains tested the MICs of FIN compared to CIP at both, pH 5.8 and pH 7.2, are lower by 2 to 8 and 2 to 4 dilution steps, respectively. Except for *qnrS*, FIN MICs at pH 5.8 do not exceed 2 µg/ml, while MICs of CIP for all mutants carrying at least one transferable resistance determinant range from 2 to 512 µg/ml. The presence of *qepA* resulted in MIC increases of 5-8 and 0-1 dilution steps at pH 5.8, and of 4-6 and 0-1 at pH 7.2, for CIP and FIN, respectively.

Conclusions:
Finafloxacin is a promising novel FQ with excellent potency at pH 5.8 and 7.2 against *E. coli* mutants expressing all known FQ resistance determinants alone and in combinations. Moreover, in contrast to CIP, FIN appears to be a poor substrate for QepA and AAC6'-Ib-cr.

Introduction

The predominant mechanisms of resistance to FQ are the mutational alteration of chromosomal target genes *gyrA/B* and *parC/E* encoding DNA gyrase and topoisomerase IV, respectively (1) and increased expression of multiple-drug resistance (MDR) efflux pumps often due to inactivation of the repressor MarR (2). Recently, plasmid-mediated horizontally transferable FQ resistance genes *qepA* (efflux pump), *qnr* (target protection) and *aac(6')-Ib-cr* (a new variant of the aminoglycoside acetyltransferase capable of modifying C7-piperazinyl-FQs, like ciprofloxacin and norfloxacin) have been described (3,4).

Due to the rapid development of bacterial resistance to clinically used FQs, novel FQs refractory to the known mechanisms of resistance are urgently needed.

Finafloxacin (Figure 1) is a novel 8-cyano-fluoroquinolone that exhibits enhanced antibacterial activity against a broad spectrum of pathogens over a pH range of 5.0 to 7.2, under which other FQs exhibit significantly reduced activity, (5). The present study was performed to determine the impact of known FQ resistance mechanisms on the *in vitro* activity of FIN at different pH in comparison with ciprofloxacin (CIP), moxifloxacin (MOX) and levofloxacin (LEV).

Methods

Cloning of *qepA*, *qnrA1* and *aac(6')-Ib-cr*:

The *qepA* gene isolated from plasmid pHPA of clinical isolate *E. coli* C316 and cloned as *Sac*I and *Sal*I fragment into pSTV28 was kindly provided by Kunikazu Yamane (6).

The *qnrA1* gene from pMG252 (kindly provided by L. Martinez-Martinez) was amplified by PCR using primers 5´-ATACAAGCTTCGGCAGTTAAAATTGGGGCT-3' and 5´-ATACAAGCTTGACCAGACTGCATAAGCAACAC-3' (7) digested with *Hind*III and ligated to the *Hind*III site of pUC19. The *aac(6')-Ib-cr* gene was amplified from a clinical isolate by PCR using primers 5´-ATACAAGCTTGATGACTGAGCATGACCTTGC-3' and 5´-ACTATAAGCTTTTAGGCATCACTGCGTGTTC-3', digested with *Hind*III and ligated into the *Hind*III site of pUC19. This strategy placed both genes under the control of the P_{lac} promoter, which can be induced by isopropyl-1-thio-β-D-galactopyranoside (IPTG).

Transformation:

Plasmids pSTV28::qepA, pUC19::qnrA1 and pUC19::aac(6')-Ib-cr were individually transfered by transformation into *E. coli* WT and its different isogenic mutants carrying additional mutations in chromosomal genes *gyrA*, *parC*, and *marR*.

Susceptibility testing:

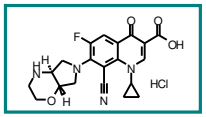
MICs of FIN, CIP, LEV and MOX were determined at pH 5.8 and 7.2 for all strains. The MICs of KAN were determined for the strains containing *aac(6')-Ib-cr* to confirm expression of *aac(6')-Ib-cr* gene. The expression of *aac(6')-Ib-cr* was induced with IPTG at a final concentration of 1mM.

Results and Discussion

Impact of chromosomal mutations in *gyrA, parC*, and *marR* on FQ susceptibility:

- Results are summarized in Table 1.
- While the relative increases in the MICs for the FQs tested were comparable (± 1 serial dilution step), the MICs of FIN determined at pH 5.8 were lower by a factor of 4 - 16 compared to CIP, MOX and LEV for parent WT mutants: WT + *gyrA*83, WT + *gyrA*83 + Δ*marR* and WT + Δ*marR* + *gyrA*87 + *parC*80.
- Compared to the other FQs, finafloxacin exhibits excellent activity at slightly acidic pH of 5.8.

Figure 1: Finafloxacin hydrochloride (FIN)



Results and Discussion

Table 1: MICs (µg/ml) of FIN, CIP, LEV and MOX for *E. coli* WT and its isogenic FQ resistant derivatives carrying combinations of chromosomal FQ resistance mutations

E. coli strain with resistance determinant	MIC [µg/ml]							
	CIP		FIN		LEV		MOX	
	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2
---- (WT)	0.06	0.015	0.015	0.03	0.25	0.015	0.25	0.015
+ *gyrA83*	2	0.25	0.5	0.5	4	0.25	4	0.25
+ *gyrA83* + *marR*Δ*175bp*	16	1	2	2	16	1	≥16	2
+ *marR*Δ*74bp*	0.5	0.03	0.125	0.25	1	0.125	1	0.06
+ *gyrA87* + *parC80*	0.5	0.06	0.25	0.25	1	0.125	4	0.25

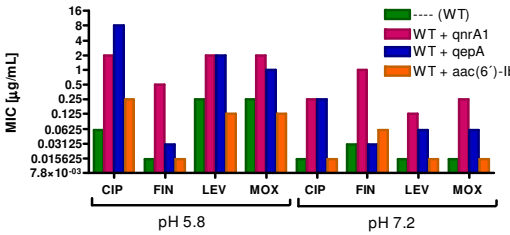
Impact of transferable resistance determinants *qnrA1, qepA*, and *aac(6')-Ib-cr* on FQ susceptibility:

- Results are summarized in Table 2. The effect of *qnrA1, qepA*, and *aac(6')-Ib-cr* expression in WT is also shown in Figure 2.
- The presence of *qnrA1* resulted in MIC increases by factors of 2 - 64 (CIP), 4 - 32 (FIN), 1 - 8 (LEV) and at 1 - 8 (MOX) at pH 5.8 and of 4 - 64 (CIP), 16 - 64 (FIN), 2 - 32 (LEV) and 2 - 32 (MOX) at pH 7.2.
- The presence of *qepA* resulted in MIC increases by factors of 32 - 128 (CIP), 1 - 2 (FIN), 4 - 8 (LEV) and 2 - 16 (MOX) at pH 5.8 and by factors of 16 - 64 (CIP), 0.5 - 2 (FIN), 2 - 4 (LEV) and 0.5 - 8 (MOX) at pH 7.2, respectively.
- The presence of *aac(6')-Ib-cr* in the isogenic mutants resulted in MIC increases by factors of 1 - 4 at pH 5.8 and pH 7.2 for CIP, while the influence on the MICs of FIN, LEV and MOX, which do not have a piperazinyl substituent at position C-7 was ≤1 serial dilution step. In the presence of the *aac(6')-Ib-cr* the MICs of KAN were higher by a factor of 4 - 8 compared to the strains without *aac(6')-Ib-cr* (Data not shown).

Table 2: MICs (µg/ml) of FIN, CIP, LEV and MOX for *E. coli* WT and its isogenic FQ resistant derivatives carrying plasmid-mediated FQ resistance determinants *qnrA1*, *qepA* and *aac(6')-Ib-cr*

E. coli strain with resistance determinant	MIC [µg/ml]							
	CIP		FIN		LEV		MOX	
	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2
WT + *qnrA1*	2	0.25	0.5	1	2	0.125	2	0.25
WT + *qepA*	8	0.25	0.03	0.03	2	0.06	1	0.06
WT + *aac(6')-Ib-cr*	0.25	0.015	0.015	0.06	0.125	0.015	0.125	0.015
WT + *gyrA83* + *qnrA1*	4	1	2	8	4	0.5	4	1
WT + *gyrA83* + *qepA*	128	8	0.5	0.5	16	1	16	0.5
WT + *gyrA83* + *aac(6')-Ib-cr*	8	0.25	1	1	4	0.25	4	0.25
WT + *gyrA83* + *marR*Δ*175bp* + *qnrA1*	64	4	8	32	64	4	64	4
WT + *gyrA83* + *marR*Δ*175bp* + *qepA*	512	32	2	4	64	4	64	4
WT + *gyrA83* + *marR*Δ*175bp* + *aac(6')-Ib-cr*	16	2	2	4	8	1	8	2
WT + *marR*Δ*74bp* + *qnrA1*	16	1	2	4	8	1	8	2
WT + *marR*Δ*74bp* + *qepA*	32	2	0.125	0.125	8	0.25	4	0.5
WT + *marR*Δ*74bp* + *aac(6')-Ib-cr*	1	0.125	0.125	0.25	1	0.06	1	0.06
WT + *gyrA87* + *parC80*+ *qnrA1*	32	4	8	16	8	4	32	8
WT + *gyrA87* + *parC80*+ *qepA*	64	1	0.5	0.5	8	0.25	8	0.5
WT + *gyrA87* + *parC80*+ *aac(6')-Ib-cr*	2	0.125	0.25	0.5	1	0.125	4	0.25

Figure 2: MICs (µg/ml) of FIN, CIP, LEV and MOX for *E. coli* WT and its isogenic FQ resistant derivatives carrying QnrA1, QepA and AAC (6')-Ib-cr



Conclusions

- Under acidic conditions (pH 5.8), finafloxacin was more active than CIP, LEV and MOX against QnrA1 and QepA expressing strains, alone, or in combination with chromosomal mutations.
- Finafloxacin was the only FQ that was not affected by expression of QepA.
- Due to the absence of a piperazinyl substituent, AAC(6')-Ib-cr under acidic conditions exhibited no effect on finafloxacin, LEV or MOX, whereas the activity of CIP was reduced.
- In general, finafloxacin exhibited excellent potency at pH 5.8 and 7.2 against *E. coli* mutants expressing all known FQ resistance determinants alone and in combinations.
- Finafloxacin promises high potency at low pH, so it is a hopeful antimicrobial agent for the treatment of infections in acidic environments like SSSI (skin and skin structure infection), complicated UTI, intraabdominal infection, infections of cystic fibrosis patients and *H. pylori* eradication.

Acknowledgement

The work of Nadine-Christine Emrich is supported by a PhD grant of JÜRGEN MANCHOT foundation, Düsseldorf, Germany.

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Cellular accumulation and intracellular activity of finafloxacin (FNX), a novel fluoroquinolone (FQ) with enhanced activity at acid pH, against *S. aureus* (*S.a.*) and *L. pneumophila* (*L.p.*): comparison with ciprofloxacin (CIP) and moxifloxacin (MXF).

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Abstract

Background. FNX, in contrast to other FQ, shows markedly lower MIC's at acidic pH (Kresken et al, ICAAC 2008, poster F1-2037). We have measured its cellular accumulation and assessed its intracellular activity against organisms located in acidic (*S.a.*, pH 5-5.5 [phagolysosomes]) or moderately acidic (*L.p.*; pH 6 [phagosomes]) organelles in human THP-1 macrophages, in comparison with CIP and MXF.

Methods. Accumulation: uninfected cells exposed to neutral and acidic pH, and cells exposed to 10 mM NH_4Cl (to neutralize the phagolysosomal pH). Intracellular activity: in infected cells (*S.a.* ATCC 25923; *L.p.* ATCC 33153) exposed to antibiotic concentrations from 0.001 to 100 mg/L for 24 h (*S.a.*) or 48 h (*L.p.*), with changes in $\log_{10}$ CFU (compared to time 0) used to fit a Hill equation to determine static (C_s) and 1 $\log_{10}$ CFU drop (C_{90}) concentrations, and maximal relative efficacy [E_{max}] (Barcia-Macay et al, AAC 50:841-851).

Results. The main results are shown in the Table.

	FNX		CIP		MXF	
Cell. Accum.	**pH 7.4**	**pH 5.5**	**pH 7.4**	**pH 5.5**	**pH 7.4**	**pH 5.5**
Control (Cc/Ce) [a]	1.9 ± 0.1	8.1 ± 1.3	9.3 ± 0.1	5.1 ± 0.8	5.5 ± 1.7	1.6 ± 0.1
+ NH4Cl (% of control)	39 ± 29	N.D.	229 ± 15	N.D.	256 ± 6	N.D.
Intr. Activity *	***S.a.***	***L.p.***	***S.a.***	***L.p.***	***S.a.***	***L.p.***
C_s (mg/L) [b]	1.8	0.08	0.26	0.35	0.2	N.D.
C_{90} (mg/L) [c]	7.5	0.38	1.00	3.59	0.54	0.93
E_{max} [d]	-1.6 ± 0.3	-2.7 ± 0.3	-1.7 ± 0.1	-2.0 ± 0.3	-2.2 ± 0.1	-2.9 ± 0.3

[a] Cellular to extracellular concentration ratio (Cc/Ce)
[b] concentration (mg/L) causing no apparent change from post-phagocytosis inoculum
[c] concentration (mg/L) causing a 1 $\log_{10}$ drop from post-phagocytosis inoculum
[d] change in $\log_{10}$ CFU per mg of cell protein from the original, post-phagocytosis inoculum for an infinitely large concentration of antibiotic (Hill equation, $R^2 > 0.95$ for all conditions)
* MIC (mg/L; broth): S. a. pH 7.4/pH 5.5 – FNX: 0.06/0.007; CIP: 0.125/1; MXF: 0.03/0.06

Conclusions. Although displaying lower MICs in acidic medium, FNX showed larger Cs and C_{90} than CIP and MXF against intracellular *S.a.* Conversely, FNX was more active against intracellular *L.p.* The divergent effect of NH4Cl on accumulation suggests distinct pH-dependent transport behaviors and / or different subcellular localizations. The data show that, even within a pharmacological class, intracellular activity cannot be predicted based on intrinsic potency and/or accumulation only, but that other parameters (subcellular disposition, localization of the bacteria) may also be critical.

This poster will be made available for download after the meeting:
http://www.facm.ucl.ac.be/posters.htm

Background and aim

Treatment of intracellular infections requires that antibiotics enter cells and express activity therein. Acidic conditions prevailing within the phagolysosomes (pH 5.0 – 5.5) or phagosomes (pH 6.0) of infected cells may, however, significantly impair the activity of many antibiotics such as gentamicin, azithromycin or clindamycin.[1-3]

Finafloxacin is a novel fluoroquinolone showing enhanced activity at acidic pH. [4] In this context, our aim was to measure assess the cellular accumulation of finafloxacin in macrophages and to measure its intracellular activity towards S.a (phagolysosomes) and L.p. (phagosomes).

Methods

Cells. Experiments were performed with human THP-1 cells, a myelomonocytic cell line displaying macrophage-like activity.[5]

Assay of cell-associated antibiotics. Fluoroquinolones were assayed by the disc-plate method using *S. aureus* ATCC 25923 as test organism or fluorimetry (for ciprofloxacin and moxifloxacin only).

Bacterial strain and susceptibility testing. *S. aureus* strain ATCC 25923 and *L. pneumophila* strain ATCC 33153 (Manassas, VA) were used thorough. MICs determinations were made, respectively, in Mueller Hinton broth (for *S.a.*, 24 h) or in α-ketoglutarate Buffered Yeast Extract broth adjusted at pH 6.9 (for *L. p.*, 48 h).

Cell infection and determination of the intracellular activities of antibiotics. Phagocytosis was initiated at a bacteria per macrophage ratio of 4 (for *S.a.*, 1 h) or 10 (for *L.p.*, 2 h), followed by elimination of non-phagocytosed bacteria by exposing the cells to 50 mg/L gentamicin (30-45 min). Cells were then transferred to fresh medium supplemented with increasing concentrations of antibiotics. Results, expressed as the change in the intracellular inoculum at 24 h (for *S. a.*) or 48 h (for *L.p.*) compared to time 0, were used to fit a Hill equation to allow determination of the values of key pharmacological descriptors of antibiotic activity (see ref. 2 for details).

References

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Results

Cellular accumulation of fluoroquinolones

A. Influence of the pH medium

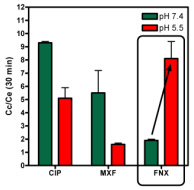

Fig. 1. Influence of the pH medium (7.4 vs. 5.5) on the cellular accumulation of finafloxacin and comparators in THP-1 cells (37°C, 30 min). Results were expressed as cellular to extracellular concentration ratio (Cc/Ce).

➔ **Decreasing the pH of the culture medium from 7.4 to 5.5 markedly enhanced the cellular accumulation of finafloxacin, with an opposite effect for CIP and MXF.**

B. Influence of ammonium chloride

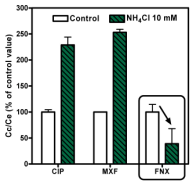

Fig. 2. Influence of ammonium chloride **(known to neutralize the phagolysosomal pH)** on the cellular accumulation of finafloxacin and comparators (37°C, 2 h). Results were expressed as a change from control. percentage of control value.

➔ **Exposure of cells to ammonium chloride reduced the cellular accumulation of finafloxacin, and had the opposite effect on CIP and MXF**

Susceptibility testings

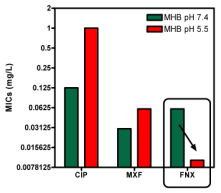

Fig. 3. Influence of pH on the intrinsic activity of finafloxacin and comparators against *S. aureus* ATCC 25923.

➔ **Decreasing the pH from 7.4 to 5.5 significantly increased the activity of finafloxacin towards *S. aureus* while the activity of CIP is markedly and that of MXF slighly reduced.**

Intracellular activity of antibiotics

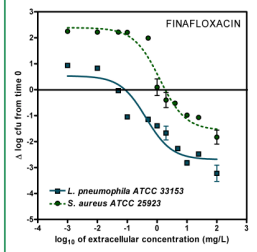

Fig. 4. Dose-response curves of finafloxacin against *S. aureus strain* ATCC 25923 (phagolysosomial infection) or *L. pneumophila* strain ATCC 33153 (phagosomal infection) phagocytized by human THP-1 macrophages after 24 h (for *S.a.*) or 48 h (for *L.p.*) incubation of the cells in the presence of increasing concentrations of antibiotic. The ordinate shows the change in CFU (in $\log_{10}$ units) per mg of cell protein.

➔ **Finafloxacin shows larger static conc. and C_{90} than CIP or MXF against intracellular *S. aureus*, while higher activity was observed towards intracellular *L. pneumophila*.**

Table 1. Pharmacological descriptors of antibiotic activity of the dose-response curves of finafloxacin (FNX), ciprofloxacin (CIP), and moxifloxacin (MFX)and against *S.a.* or *L.p.*

Parameters	*S.a.*			*L.p.*		
	FNX	**CIP**	**MXF**	**FNX**	**CIP**	**MXF**
Cs (mg/L) [a]	1.8	0.26	0.20	0.08	0.35	N.D.
C_{90} (mg/L) [b]	7.5	1.00	0.54	0.38	3.59	0.93
E_{max} [c]	-1.6 ± 0.3	-1.7 ± 0.1	-2.2 ± 0.1	-2.7 ± 0.3	-2.0 ± 0.3	-2.9 ± 0.3

[a] concentration (mg/L) causing no apparent change from post-phagocytosis inoculum (static concentration)
[b] concentration (mg/L) causing a 1 $\log_{10}$ drop from post-phagocytosis inoculum
[c] change in $\log_{10}$ CFU per mg of cell protein from the original, post-phagocytosis inoculum for an infinitely large concentration of antibiotic (Hill equation, $R^2 > 0.95$ for all conditions)

Conclusions

Despite its higher activity in acidic medium (broth), finafloxacin was less potent (larger Cs and C_{90} values) than ciprofloxacin or moxifloxacin towards *S. aureus* (phagolysosomial infection; pH ~ 5-5.5). In contrast, finafloxacin is more potent towards *L. pneumophila* (phagosomal infection; pH ~ 6). This is not consistent with accumulation levels or MIC data (neutral and acidic pH) and suggests that, even within a given pharmacological class, the level of intracellular activity cannot be predicted from intrinsic potency (MIC) and/or cellular pharmacokinetics only, but that other parameters not analyzed here are critical.

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Activity of the Investigational Fluoroquinolone Finafloxacin and Seven Other Antimicrobial Agents Against 83 Obligately Anaerobic Bacteria.

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Abstract

Background: Finafloxacin (FIN) is a novel fluoroquinolone belonging to a 8-cyano subclass and exhibits enhanced activity at slightly acidic pH. FIN exhibited superior activity to comparator fluoroquinolones in a wide range of rodent infection models. With the present study the activity of FIN against 83 recently isolated strains of obligately anaerobic bacteria including reference strains was tested and compared to various other antimicrobials.
Methods: FIN was compared with moxifloxacin (MOX), levofloxacin (LEV), ciprofloxacin (CIP), clindamycin (CLI), imipenem (IMP), piperacillin/tazobactam (PIT) and metronidazole (MET) against 62 strains of the *Bacteroides fragilis* group and 21 *Clostridium difficile* strains. MICs were determined employing the microdilution technique in Wilkens-Chalgren broth supplemented with vitamin K1 and haemin.
Results: The MIC_{50} and MIC_{90} values (µg/ml) are listed in the Table.
Conclusions: FIN has promising activity against several pathogenic species of the *B. fragilis* group and is slightly more active than MOX against the obligately anaerobic bacteria tested here. Further work will be directed towards investigating the anti-anaerobic activity of finafloxacin under acidic conditions.

Introduction

Finafloxacin (FIN; Fig. 1) is a novel fluoroquinolone belonging to a 8-cyano subclass and exhibits enhanced activity at slightly acidic pH. FIN exhibited superior activity to comparator fluoroquinolones in a wide range of rodent infection models. With the present study the activity of FIN against 83 recently isolated strains of obligately anaerobic bacteria including reference strains was tested and compared to various other antimicrobials.

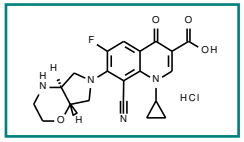

Figure 1 Finafloxacin HCl

Methods

Bacterial Strains
83 obligately anaerobes including reference strains were taken from the culture collection from the Institute for Medical Microbiology and Epidemiology of Infectious Diseases, University of Leipzig, Germany. The strains were collected from clinical specimens at the Institute and from national and international studies and obtained in part from other laboratories. The following strains were used: *Bacteroides fragilis* group (n=62): *B. caccae* (5); *B. distasonis* (10); *B. eggerhtii* (4); *B. fragilis* (6); *B. merdae* (3); *B. ovatus* (7), *B. stercoris* (6); *B. thetaiotaomicron* (7); *B. uniformis* (5); *B. vulgatus* (9) and *Clostridium difficile* (n=21).

Antimicrobial Agents
Antimicrobial agents were obtained as laboratory powders of known potency: FIN from MerLion Pharmaceuticals GmbH, Berlin, Germany; metronidazole (MET) and clindamycin (CLI) from Sigma Chemical Co., St. Louis, USA; moxifloxacin (MOX) and ciprofloxacin (CIP) from Bayer Vital GmbH, Leverkusen, Germany; levofloxacin (LEV) from Aventis Pharma Frankfurt/M., Germany; imipenem (IMP) from MSD Sharp & Dohme GmbH, Haar, Germany; and piperacillin/tazobactam (PIT) from Sigma Chemical Co. and Otsuka Chemical Co. Ltd., Osaka, Japan, respectively.

Broth microdilution MIC determinations
Tests were performed according to the recommendations of the Deutsches Institut für Normung (DIN) and standard DIN 58940-83. The bacterial inocula were prepared by suspending growth from 48 hour cultures grown on supplemented Columbia blood agar in Wilkins-Chalgren broth supplemented with vitamin K_1 and haemin. After semi-automated inoculation (Dynatech MIC-2000-inoculator, Dynatech Laboratories, Inc., Chantilly, USA) resulting in a final dilution of approximately 1.0×10^5 CFU/well (1.0×10^6 CFU/ml), plates were incubated for 48 h at 37°C in an anaerobic chamber. The MIC was defined as the lowest antibiotic concentration that inhibited visible growth.
In addition, pH of broth containing FIN was determined in part before and after incubation.

Results and Discussion

Antimicrobial Agent	*B. fragilis* group (n=62)		*C. difficile* (n=21)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
FIN	0.5	2	1	16
MOX	1	2	8	16
LEV	2	8	32	>64
CIP	16	16	8	32
CLI	1	8	4	>64
IMP	0.25	1	2	4
PIT	0.5	4	0.5	2
MET	0.5	1	0.125	0.125

Table 1: MIC_{50}/MIC_{90} (µg/ml) of antimicrobials against anaerobes

Results and Discussion

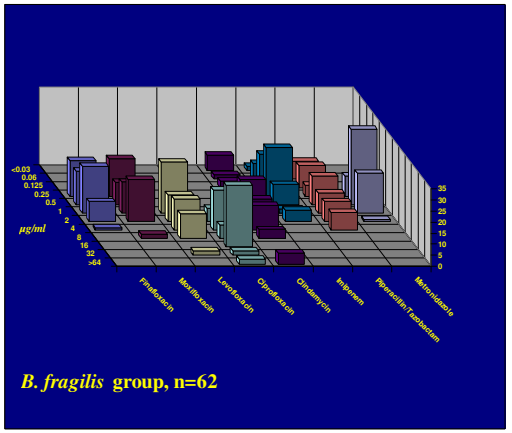

Figure 2

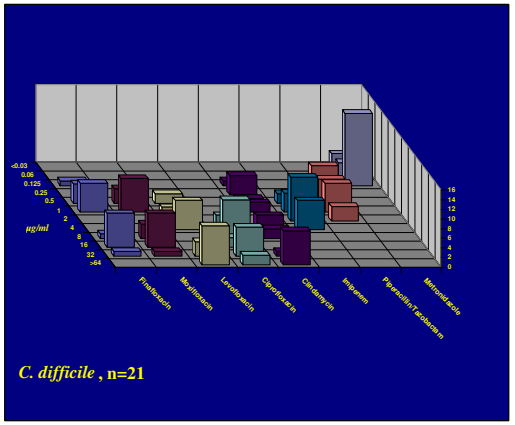

Figure 3

The Figures 2 and 3 show the scatter histograms of MIC values obtained for FIN and the seven other antimicrobial agents against 83 obligately anaerobic bacteria included in this study.

pH was approx. 7.2 before incubation and 5.0 to 7.5 after 48 h of incubation depending on the strains tested and the growth of the bacteria.

The MIC values of the *C. difficile* strains and group 3 and 4 quinolones (Figure 3) seem to display a bimodal distribution indicating that some strains are significantly less susceptible to these quinolones.

Overall, FIN was particularly active against strains of the *B. fragilis* group (Figure 2) and *C. difficile strains* (Figure 3), where it was equal to or more active than MOX and more active than LEV and CIP.

Conclusions

FIN has promising activity against a number of pathogenic species of the *B. fragilis* group and some *C. difficile* strains and is slightly more active than MOX against the obligately anaerobic bacteria tested here.
Further work will be directed towards investigating the anti-anaerobic activity of finafloxacin under acidic conditions and against additional obligately anaerobic bacteria.

Literature

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