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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:

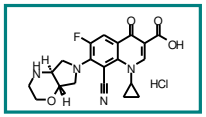
MICs of FIN, CIP, LEV and MOX were determined at pH 5.8 and 7.2 for all strains. The MICs of KAN were determined for the strains containing *aac(6')-Ib-cr* to confirm expression of *aac(6')-Ib-cr* gene. The expression of *aac(6')-Ib-cr* was induced with IPTG at a final concentration of 1mM.

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

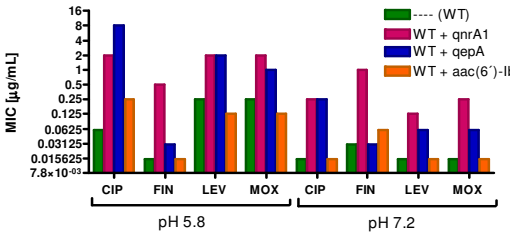
Impact of transferable resistance determinants *qnrA1, qepA*, and *aac(6')-Ib-cr* on FQ susceptibility:

- 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.

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Literature

(1) *Hooper, David C. 2001. Emerging Infectious Diseases, 7: 337-341.*
(2) *Alekshun, Michael N., Levy, Stuart B. 1997. Antimicr. Agents Chemother. 41: 2067–2075.*
(3) *Ari Robicsek, Jacob Strahilevitz, George A Jacoby, Mark Macielag, Darren Abbanat, Chi Hye Park, Karen Bush & David C Hooper. 2006. Nature Medicine 12: 83-88.*
(4) *Ari Robicsek, George A Jacoby, David C Hooper 2006. Lancet Infect Dis. 6: 629–40.*
(5) *Kresken et al., 46th ICAAC, Washington DC 2008, Poster No. F1-2037.*
(6) *Kunikazu Yamane, Jun-ichi Wachino, Satowa Suzuki, Kouji Kimura, Naohiro Shibata, Haru Kato, Keigo Shibayama, Toshifumi Konda, and Yoshichika Arakawa. 2007. Antimicr. Agents Chemother. 51: 3354–3360.*
(7) *J. M. Rodriguez-Martínez, C. Velasco, I. García, M. E. Cano, L. Martínez-Martínez and A. Pascual. 2007. Antimicr. Agents Chemother. 51: 2236–2239.*