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Activity of the Investigational Fluoroquinolone Finafloxacin against Ciprofloxacin-Sensitive and -Resistant *Acinetobacter baumannii* Isolates[▽]

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This study compared the activity of finafloxacin, a novel fluoroquinolone which shows enhanced activity under acidic pH, and that of ciprofloxacin against *Acinetobacter baumannii* under standard conditions (pH 7.2) and at a pH of 5.8. Overall, finafloxacin demonstrated superior activity to ciprofloxacin under acidic conditions. Furthermore, finafloxacin showed comparable activity to ciprofloxacin at pH 7.2. Hence, finafloxacin could be a promising new antimicrobial agent for the treatment of *A. baumannii* infections at acidic body compartments.

The continuing spread of multidrug-resistant *Acinetobacter baumannii* is of serious concern to health professionals and often leaves few therapeutic options (7). Fluoroquinolones have in the past shown good activity against *A. baumannii* (8); however, over the past decade there has been a constant rise in fluoroquinolone resistance (11). Resistance to fluoroquinolones in *A. baumannii* is mediated primarily by stepwise selection of mutations in the drug targets *gyrA* and *parC*. The first step is a *gyrA* mutation, most commonly leading to a Ser83-Leu substitution (18). This is often sufficient to render the isolate intermediate or fully resistant to ciprofloxacin. A second mutation, in *parC*, most often causing a Ser80-Leu substitution, usually leads to high-level resistance to all fluoroquinolones (17, 19). In addition, drug efflux contributes to fluoroquinolone resistance (9, 13).

Finafloxacin (Fig. 1) is a novel fluoroquinolone currently undergoing phase II clinical trials. This drug shows enhanced activity under acidic pH, where other fluoroquinolones display decreased activity. Infected body sites can have a significantly lower pH than healthy tissue (Table 1). The objective of this study was to determine the activity of finafloxacin and ciprofloxacin against *A. baumannii* isolates with characterized *gyrA* and *parC* genes under standard testing conditions and under an acidic pH.

A total of 68 previously characterized genotypically unrelated ciprofloxacin-sensitive and -resistant *A. baumannii* clinical isolates were investigated (Table 2) (9, 13, 19). Of these, 18 are wild-type GyrA/ParC, 26 have a GyrA substitution, and 24 have a double GyrA/ParC substitution. Two isolates overexpress the efflux pump *adeB* compared to their isogenic parent strains (9, 13). Ciprofloxacin and finafloxacin MICs were determined by agar dilution under standard conditions (pH 7.2) (5), and at a pH of 5.8. pH 5.8 was chosen as it is representative of a number of infected body sites (lower respiratory tract, skin, stomach mucosa) and is the mean pH of urine during urinary tract infection. Ciprofloxacin and finafloxacin powders were supplied by their respective manufacturers (Bayer AG, Wuppertal, Germany, and MerLion Pharmaceuticals GmbH, Berlin). Mueller-Hinton agar (Oxoid, Wesel, Germany) was prepared following the manufacturer's instructions, and the pH was adjusted by the addition of HCl prior to pouring antibiotic-containing plates. The pH of the agar was checked once solidified. Ciprofloxacin- and finafloxacin-containing plates were prepared on the same day using freshly prepared media, and MICs were determined simultaneously.

Results are summarized in Table 2. At normal pH (pH 7.2), finafloxacin had comparable activity to ciprofloxacin against wild-type GyrA/ParC isolates, with both having MIC_{50} and MIC_{90} values of 0.25 μg/ml and 1 μg/ml, respectively. At the lower pH, finafloxacin recorded MIC_{50} and MIC_{90} values of 0.12 μg/ml, compared to 2 μg/ml and 4 μg/ml for ciprofloxacin, respectively.

Comparable finafloxacin and ciprofloxacin activities at normal pH were also found in isolates with a GyrA substitution, with the majority of isolates recording either the same MIC or a finafloxacin MIC within one dilution of the respective ciprofloxacin MIC. However, at the lower pH, finafloxacin MIC_{50} and MIC_{90} values were both lowered from 16 μg/ml to 2 μg/ml, compared to ciprofloxacin MIC_{50} and MIC_{90} rising from 8 μg/ml and 16 μg/ml, respectively, to 32 μg/ml and >128 μg/ml, respectively.

Finafloxacin was more potent than ciprofloxacin against isolates with the double GyrA/ParC substitution tested under normal conditions, Finafloxacin recorded MIC_{50} and MIC_{90} values of 32 μg/ml and 64 μg/ml, respectively, compared to ciprofloxacin MIC_{50} and MIC_{90} values of 128 μg/ml and >128 μg/ml, respectively. Activity of finafloxacin at pH 5.8 showed an up to 16-fold lowering of MIC, with MIC_{50} and MIC_{90} values of 4 μg/ml and 8 μg/ml, respectively. In contrast, all these isolates recorded a ciprofloxacin MIC of >128 μg/ml. Two isolates which have been shown to overexpress the efflux pump AdeB also exhibited up to a 16-fold reduction in finafloxacin MIC under acidic conditions.

The effect of pH on antimicrobial activity is well known and

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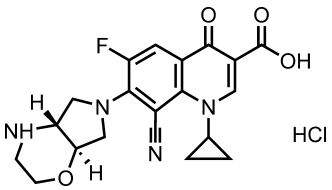

FIG. 1. Structure of finafloxacin HCl.

is dependent upon the antimicrobial agent (16). Acidification of media incubated in 5% CO_2 affects macrolide activity (10), and ciprofloxacin activity is reduced in acidic sites such as the phagolysosome (1). In the present study we found a polar effect of the fluoroquinolones ciprofloxacin and finafloxacin under acidic compared to standard conditions.

Finafloxacin has been shown to exhibit lowered MICs against a range of pathogenic bacteria under acidic conditions compared to at neutral pH, whereas ciprofloxacin and levofloxacin MICs were higher under acidic conditions (20). The mechanism of enhanced or reduced finafloxacin activity is thought to result, at least in part, from the different physiochemical properties of the fluoroquinolone molecules. Many fluoroquinolones are zwitterionic in character, and thus their lipophilicity and partitioning across biological membranes are influenced by the presence of different ionizable groups and the pH of the surroundings (6, 12, 14).

Another mechanism may be the result of changes in bacterial gene expression leading to greater drug diffusion/accumulation (more activity) or less diffusion/accumulation (reduced activity). For example, in *Serratia marcescens* the porins OmpF and OmpC are induced by alkaline and acidic conditions, respectively, while norfloxacin accumulation is reduced under acidic conditions compared to accumulation under neutral or alkaline conditions (2, 3). *A. baumannii* shows an innate resistance to many antimicrobials which is attributed to its low outer membrane permeability (15), and fluoroquinolones are known substrates of their efflux pumps (13). It is not known what effect the pH has on porin or efflux pump expression in *A. baumannii*; however, in this study, overexpression of *adeB* isolates results in lower finafloxacin MICs under acidic pH than under standard conditions. Whether this effect is due to reduced expression of the AdeB efflux pump system, due to other efflux pumps, or porin mediated remains to be determined.

In summary, finafloxacin demonstrated superior activity to ciprofloxacin under acidic conditions against ciprofloxacin-sensitive and -resistant *A. baumannii*. Furthermore, finafloxacin

TABLE 1. pH values as measured for different body fluids and tissues in the presence or absence of infection[a]

Organ/fluid	pH (range)	
	Normal	With bacterial infection
Blood	7.4	
Plasma	7.39 (7.33–7.45)	6.5
Lymph	7.4	
Lung tissue	7.3 (7.25–7.5)	6.6 (6.0–7.0)
Pleural fluid	7.2 (6.8–7.6)	6.3 (6.0–7.0)
Abdominal/peritoneal fluid	7.5 (7.0–8.4)	6.2
Cerebrospinal fluid	7.4 (7.35–7.70)	6.5
Cervical mucus	5.5	5.7
Vagina	4.36 (4.29–4.43)	4.8
Tears	7.47 (7.3–7.7)	7.0 (6.3–7.1)
Saliva	6.7 (5.6–7.6)	
Liver tissue	7.0 (6.8–7.2)	6.6 (intra-abdominal infection)
Bile	6.01 (5.98–6.72)	
Kidney tissue	7.0	
Urine	6.5–7.5	5.2 (3.5–6.0)
Skin tissue	7.0 (6.5–7.5)	6.2
Transudates	7.5 (7.45–7.68)	3.5 (2.0–6.5)
Sweat	5.0 (3.8–6.5)	
Abscess	6.9	

[a] Data were compiled from DISEASEDEX (http://www.micromedex.com/products/diseasedexgeneral/) and "Handbook of biological data" (4).

TABLE 2. Agar dilution MICs of ciprofloxacin (CIP) and finafloxacin (FIN) under normal pH (7.2) and acidic (pH 5.8) conditions against characterized *A. baumannii* isolates

Strain no.	Amino acid substitutions		MIC (μg/ml)			
	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	Ser83-Leu		4	8	32	1
23	Glu87-Gly		4	8	32	1
24–26	Ser83-Leu		4	16	32	2
27, 28	Ser83-Leu		4	16	32	1
29	Ser83-Leu		4	32	32	4
30	Ser83-Leu		8	8	32	0.5
31	Ser83-Leu		8	16	32	1
32	Ser83-Leu		16	16	32	1
33	Ser83-Leu		8	16	128	1
34, 35	Ser83-Leu		8	16	128	2
36, 37	Ser83-Leu		16	16	128	2
38	Ser83-Leu		32	16	128	2
39–42	Ser83-Leu		16	16	>128	2
43	Ser83-Leu		32	16	>128	2
44	Ser83-Leu	Glu84-Lys	32	16	>128	4
45, 46	Ser83-Leu	Ser80-Leu	64	16	>128	2
47	Ser83-Leu	Ser80-Phe	64	32	>128	4
48, 49	Ser83-Leu	Ser80-Leu	128	16	>128	2
50	Ser83-Leu	Ser80-Leu	128	16	>128	4
51–53	Ser83-Leu	Ser80-Leu	128	32	>128	2
54–56	Ser83-Leu	Ser80-Leu	128	32	>128	4
57	Ser83-Leu	Ser80-Phe	128	32	>128	4
58	Ser83-Leu	Ser80-Leu	128	64	>128	4
59	Ser83-Leu	Glu84-Lys	128	64	>128	8
60–62	Ser83-Leu	Ser80-Leu	128	64	>128	8
63	Ser83-Leu	Glu84-Lys	>128	64	>128	2
64	Ser83-Leu	Ser80-Leu	>128	64	>128	4
65	Ser83-Leu	Ser80-Leu	>128	64	>128	8
66	Ser83-Leu		32	16	128	2
67,[a] 68[a]	Ser83-Leu	Ser80-Leu	>128	64	>128	4

[a] *adeB* overexpressing; isolates 67 and 68 are clinical isolates that are otherwise identical to isolate 66 (based on pulsed-field gel electrophoresis pattern).

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showed comparable activity to ciprofloxacin at pH 7.2 and could be a promising new antimicrobial agent for the treatment of *A. baumannii* infections at acidic body compartments.

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