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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

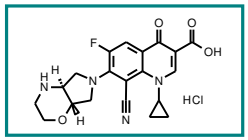
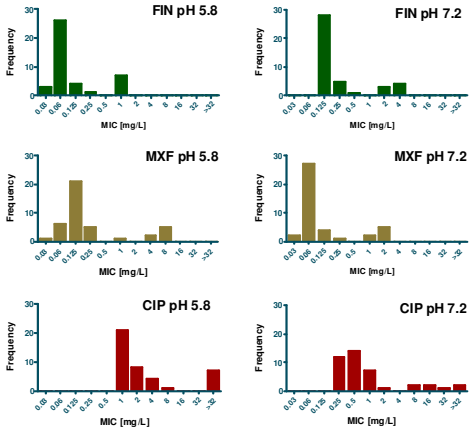


Table 2. Spontaneous resistance frequencies and mutation prevention concentration of finafloxacin, ciprofloxacin and moxifloxacin against *S. aureus* NRS-384 (USA-300) , selected at multiple drug concentrations at pH 7.2 and pH 5.8.

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.**