In vitro Evaluation of the Novel Fluoroquinolone Finafloxacin against *Yersinia pestis*

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Introduction

Yersinia pestis is the causative agent of plague, a zoonotic disease. Although it is primarily a disease of rodents, with humans being an accidental host, it has caused three pandemics, the most notorious being the Black Death in the 14th century which killed an estimated 50 million people [(1)]. Plague remains endemic in India, South East Asia, America and Africa where the emergence of multi-drug resistant strains has been recorded in Madagascar, and mortality for pneumonic plague is 100% if left untreated [(2)]. Therefore plague is still a substantial public health problem today. In the initial infection in humans, *Y. pestis* is phagocytised by macrophages where it is able to survive and replicate in the intracellular environment, before release into the extracellular environment leading to systemic spread.

Finafloxacin is a novel fluoroquinolone shown to have increased antibacterial activity at acidic pH [(3)]. Other fluoroquinolones have reduced antibacterial activity at lower pH and therefore finafloxacin may offer greater efficacy in the treatment of intracellular infections, where the local site of infection is acidic [(4)].

Methods

Finafloxacin was supplied to Dstl by MerLion Pharmaceuticals Pte Ltd. Ciprofloxacin was obtained from Sigma-Aldrich (UK).

The minimum inhibitory concentrations (MICs) of finafloxacin and ciprofloxacin were determined for *Y. pestis* strains CO92 and GB, using the broth micro dilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [(5)]. Assays were performed in 96 well plates in Blood Agar (BA) broth and incubated at 28 °C for 24 hours. Each assay was performed in triplicate and repeated three times in media adjusted to pH 6 or pH 7. The minimum bactericidal concentrations (MBCs) for finafloxacin and ciprofloxacin were determined by plating 100 µL aliquots from the MIC dilutions showing no visible bacterial growth onto Congo Red (CR) agar, in triplicate. The plates were incubated for 72 hours at 28 °C. The MBC was recorded as the lowest concentration of antibiotic that killed 99.9% of the bacteria in the original inoculum.

Time kill assays were performed by preparing antibiotic solutions of finafloxacin or ciprofloxacin at 4 times the MIC in 10 mL of BA broth adjusted to pH 6 or pH 7. Broths were inoculated with *Y. pestis* at a concentration of approximately 5 x 10^5 CFU/mL; untreated control bacteria at the same concentration grown in the absence of antibiotic were compared. All samples were incubated shaking at 180 rpm at 28 °C. Samples were taken at 0, 2, 4, 6 and 24 hours, serial diluted and plated out onto CR agar. Plates were enumerated after incubation at 28 °C for 72 hours. A bactericidal effect was defined as a 3 log 10 reduction or greater in CFU/mL compared with the original inoculum. All samples were performed in duplicate and each assay was performed three times.

Purpose

The aim of this study was to evaluate the *in vitro* activity of finafloxacin, in comparison to ciprofloxacin, against *Y. pestis* in neutral and acidic pH conditions.

Results

MICs and MBCs

- At pH 7 finafloxacin has comparable inhibitory and bactericidal activity to ciprofloxacin.
- At pH 6 finafloxacin has increased inhibitory and bactericidal activity in comparison to ciprofloxacin (Tables 1 and 2).

Organism	Minimum inhibitory concentration (µg/mL)			
	Finafloxacin		Ciprofloxacin	
	pH 6	pH 7	pH 6	pH 7
Y. pestis CO92	0.25	0.5	4	0.25
Y. pestis GB	0.25	0.5	2	0.25

Table 1. Minimum inhibitory concentrations (µg/mL) of finafloxacin and ciprofloxacin for *Y. pestis*.

Organism	Minimum bactericidal concentration (µg/mL)			
	Finafloxacin		Ciprofloxacin	
	pH 6	pH 7	pH 6	pH 7
Y. pestis CO92	0.25	0.5	32	0.25
Y. pestis GB	0.25	0.5	64	0.5

Table 2. Minimum bactericidal concentrations (µg/mL) of finafloxacin and ciprofloxacin for *Y. pestis*.

Time Kill Assays

- Both ciprofloxacin and finafloxacin showed similar activity against both strains of *Y. pestis* at pH7, with bactericidal action over a 24 hour period (Figures 1 and 2).
- Similarly, at pH 6, both ciprofloxacin and finafloxacin demonstrated bactericidal activity over a 24 hour period against both strains of *Y. pestis* (Figures 1 and 2); however, finafloxacin demonstrated a faster rate of kill than ciprofloxacin, showing a 3 log 10 reduction in bacteria within 2 hours, whereas the same reduction was not seen with ciprofloxacin until 24 hours.
- At pH 6 finafloxacin demonstrated complete kill of both strains of *Y. pestis* over a 24 hour period.

References

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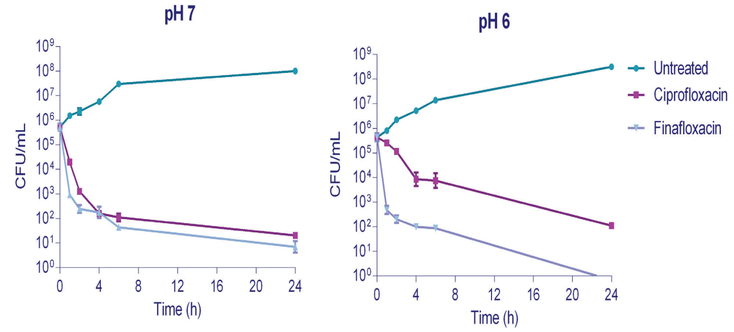

Figure 1. Time kill of antibiotics against *Y. pestis* strain CO92 in BA broth adjusted to pH 7 or pH6.

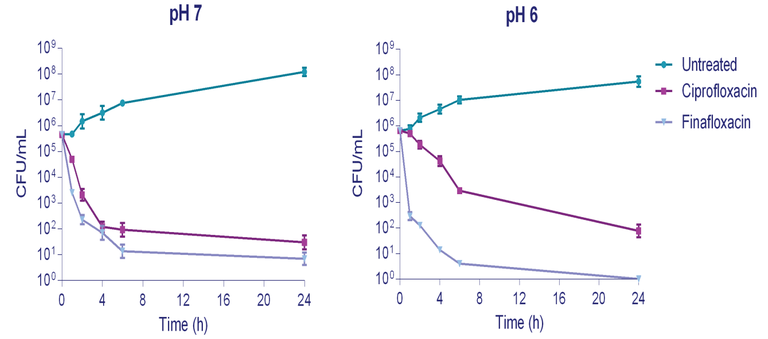

Figure 2. Time kill of antibiotics against *Y. pestis* strain GB in BA broth adjusted to pH 7 or pH 6.

Conclusions

- Under neutral pH conditions finafloxacin and ciprofloxacin demonstrate comparable inhibitory and bactericidal activity against *Y. pestis*.
- Promisingly, finafloxacin showed increased bactericidal activity at an acidic pH, in comparison to ciprofloxacin, against 2 strains of *Y. pestis*, with complete kill over a 24 hour period. Demonstrating finafloxacin has improved *in vitro* activity over ciprofloxacin in acidic conditions.
- In the initial stages of infection with *Y. pestis* the bacteria reside and replicate in the intracellular environment, where the pH in organelles is often acidic [(4)]; therefore the increased bactericidal activity of finafloxacin in these conditions may enable increased efficacy compared to ciprofloxacin when used as prophylaxis against *Y. pestis*.
- Thus, further study of finafloxacin for efficacy against *Y. pestis in vivo* is warranted.