Evaluation of *in vitro* activity of Delafloxacin against contemporary *Neisseria gonorrhoeae* isolates

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Revised Abstract

Background
Neisseria gonorrhoeae (Ng) has developed resistance to all classes of antimicrobials previously recommended for treatment of gonorrhea; today, only one cephalosporin (ceftriaxone) is recommended by the CDC as first-line regimen for treatment of all gonococcal infections, and clinical isolates with reduced susceptibility to it have been observed. As such, new antimicrobials to treat gonorrhea are urgently needed. Previously, delafloxacin (DLX), a unique investigational fluoroquinolone, demonstrated excellent *in vitro* activity against ciprofloxacin-susceptible and –resistant clinical strains of Ng. The present studies were undertaken to evaluate DLX against recent clinical isolates of Ng, including those with decreased susceptibility to cephalosporins.

Methods
For both studies, agar dilution antimicrobial susceptibility testing was performed using the CDC Gonococcal Isolate Surveillance Project (GISP) protocol and the Clinical Laboratory Standards Institute (CLSI) criteria. In study 1 (UW), DLX and comparators were tested against 50 ciprofloxacin-resistant (MICs 1 – >16 µg/mL) Ng strains collected from 2012 – 2013 and 7 control strains, along with the ranges (in µg/mL) tested: DLX, 0.002 – 16; penicillin, 0.03 – 16; tetracycline, 0.03 – 16; spectinomycin, 32 – 128; ceftriaxone, 0.001 – 2; cefixime, 0.001 – 2; ciprofloxacin, 0.002 – 64; azithromycin, 0.015 – 16. In study 2 (UAB), DLX and the following comparators were tested against 100 Ng strains collected from 2012 – 2013 and 7 control strains, along with the ranges (in µg/mL) tested: DLX, 0.0005 – 8; penicillin, 0.25 – 32; spectinomycin, 128; ceftriaxone, 0.008 – 1; cefixime, 0.015 – 1; ciprofloxacin, 0.015 – 16; azithromycin, 0.03 – 32. Ciprofloxacin MICs in study 2 ranged from ≤0.015 – 16; 35 of these isolates were ciprofloxacin-resistant (MICs ≥ 1 µg/mL).

Results
DLX was highly active against Ng in both panels, with MIC ranges of 0.004 – 0.25 (UW) and ≤0.0005 – 0.06 µg/mL (UAB). The MIC_{50} and MIC_{90} for DLX in study 1 were 0.06 and 0.125 µg/mL; for study 2, they were 0.001 and 0.06 µg/mL.

Conclusions
DLX shows robust *in vitro* activity against contemporary clinical isolates of Ng with different resistance profiles. These results indicate that DLX might be an effective novel treatment option for gonorrhea.

Introduction

Gonorrhea is the second most reported notifiable infectious disease in the US, with more than 300,000 cases in 2012 alone (1). The range of infections caused by *N. gonorrhoeae*, spread via sexual contact includes urethritis, cervicitis, pharyngitis, pelvic inflammatory disease. However, in many cases, particularly cervical, pharyngeal and rectal infections, the disease is asymptomatic. If untreated, gonorrhea can lead to serious reproductive issues in women, including pelvic inflammatory disease, ectopic pregnancy and infertility. In addition, there is the more dire concern that gonococcal infections can facilitate HIV transmission (1). Currently, gonorrhea is treated with empiric antimicrobial therapy, as susceptibility testing is not routinely available in US clinics (1, 2). As such there is a pressing need for agent(s) that can be safely administered as effective, single dose antimicrobial for treatment gonorrhea.

The growing resistance of *N. gonorrhoeae* to antibiotics has accelerated over the past 70 years (Figure 1). The Gonococcal Isolate Surveillance Project (GISP) was started by the CDC in 1986 to monitor gonococcal antimicrobial susceptibility. Early antibiotics such as sulfonamides and penicillin were very effective against this organism; however, resistance has diminished their curative abilities. Eventually tetracycline also lost its effectiveness against this organism, and during the 1990s and 2000s, fluoroquinolone-resistant *N. gonorrhoeae* (QRNG) emerged (1, 2, 3, 4). Their rapid spread led to QRNG rising to >5% of *N. gonorrhoeae* isolates in the US; as such, as of 2007 fluoroquinolones could no longer be recommended for treating gonorrhea (3), falling short of the recognized standard of a >95% effectiveness rate. Only the cephalosporins are recommended for anti-gonococcal therapy (1, 2). The current treatment regimen, 250 mg IM of ceftriaxone plus either a single dose of azithromycin 1 g orally or a 7-day course of doxycycline 100 mg (to treat *Chlamydia trachomatis*, a frequently-occurring co-pathogen) has been effective, but the increase in ceftriaxone-resistant *N. gonorrhoeae* in the last decade has underscored the need for new agents to treat gonorrhea. The purpose of this study was to determine whether the investigational fluoroquinolone, delafloxacin, has the anti-gonococcal activity to warrant clinical evaluation

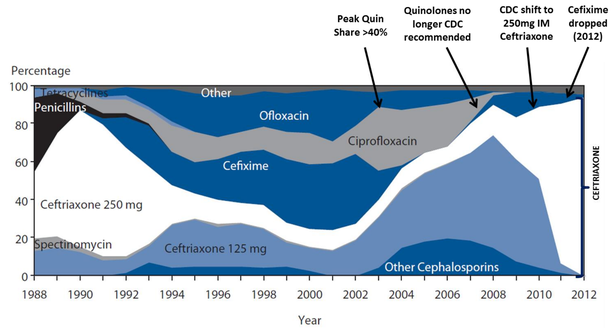


Figure1. Time course of prescribing patterns for *N. gonorrhoeae* infections, 1988 – 2012. Source: Centers for Disease Control

Methods

Bacterial strains
For study 1 (UW), agar dilution susceptibility testing against fifty ciprofloxacin-resistant (MICs 1 – 32 µg/mL) clinical *N. gonorrhoeae* strains and seven CDC GISP control strains (F-18/ATCC49226, F-28, P681E, SPJ-15, SPL-4, CDC 10328, and CDC10329) was performed at the Neisseria Reference Laboratory, University of Washington. The strains of *N. gonorrhoeae* were isolated from various clinical sources (43 urethral, 2 pharyngeal, 3 rectal, 1 vaginal, and 1 endocervical) collected between 2012 and 2013 from patients in seven US cities.
In study 2 (UAB), 100 isolates that had been collected between 2012 and 2013 from the GISP, plus the same seven QC strains used in study 1 were tested.

Antimicrobial Susceptibility Testing
For both studies, agar dilution antimicrobial susceptibility testing was performed using the CDC GISP protocol and the Clinical Laboratory Standards Institute (CLSI) criteria. In study 1, delafloxacin and comparator drugs were used in the following ranges (in µg/mL): delafloxacin, 0.002 – 16; penicillin, 0.03 – 16; spectinomycin, 32 - 128; tetracycline, 0.03 – 16; ceftriaxone, 0.001 – 2; cefixime, 0.001 – 2; ciprofloxacin, 0.002 – 64; azithromycin, 0.015 – 16. In study 2, delafloxacin and the following comparators were used in the following ranges (in µg/mL): delafloxacin, 0.0005 – 8; penicillin, 0.25 – 32; spectinomycin, 128; ceftriaxone, 0.008 – 1; cefixime, 0.015 – 1; ciprofloxacin, 0.015 – 16; azithromycin, 0.03 – 32. Ciprofloxacin MICs in study 2 ranged from ≤0.015 – 16; 35 of these isolates were ciprofloxacin-resistant (MICs ≥ 1 µg/mL).

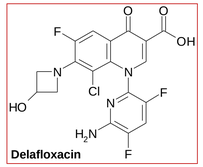
Results – Study 1

In study 1, delafloxacin was highly active against recent clinical isolates of ciprofloxacin- and multidrug-resistant *N. gonorrhoeae* (Figures 2 and 3).

- **The MIC range, MIC_{50}, and MIC_{90} of delafloxacin in this study were 0.004 – 0.25, 0.06, and 0.125 µg/mL, respectively.**
- **The MIC_{50} and MIC_{90} (16 µg/mL) for ciprofloxacin against this collection of isolates were 256 and 128-fold higher, respectively, than those of delafloxacin (Table 1).**
- **The highest delafloxacin MIC observed in this study was 0.25 µg/mL, whereas that for ciprofloxacin was 32 µg/mL.**
- **In this study, resistance to ciprofloxacin does not correlate with increased delafloxacin MICs (Figure 3).**
- **37 (74%) of the strains in this study were multidrug-resistant.**

Table 1. *in vitro* Activity Against Contemporary, Cipro-resistant Clinical Isolates from UW (Study 1)

Compound	MIC Range	MIC_{50}	MIC_{90}
Penicillin	0.125 - >16	2	8
Tetracycline	0.5 - >16	4	>16
Spectinomycin	Susceptible		
Cefixime	0.008 - 0.25	0.06	0.25
Ceftriaxone	0.004 - 0.125	0.03	0.125
Ciprofloxacin	1 - 32	16	16
Delafloxacin	0.004 - 0.25	0.06	0.125
Azithromycin	0.03 - 1	0.25	0.5



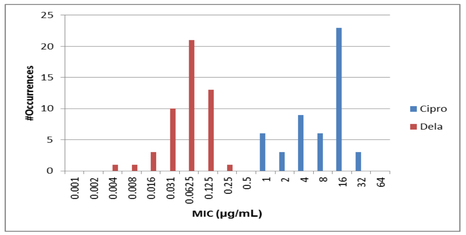


Figure 2. MIC range and distribution for delafloxacin and ciprofloxacin in Study 1.

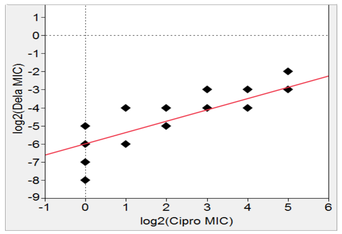


Figure 3. Delafloxacin activity, in log-2 scale, as a function of ciprofloxacin activity in Study 1. The hashed lines correspond to an MIC=1 µg/mL, which sets the bar for ciprofloxacin resistance.

Results – Study 2

In study 2, delafloxacin was highly active against the clinical isolates of *N. gonorrhoeae* that were tested (Figures 4 and 5).

- **MIC ranges were ≤0.0005-0.06 µg/mL. The MIC_{50} and MIC_{90} for delafloxacin were 0.001 and 0.06 µg/mL, respectively (Table 2).**
- **The highest delafloxacin MIC observed in this study was 0.0.0625 µg/mL, whereas that for ciprofloxacin was 16 µg/mL.**
- **In this study also, resistance to ciprofloxacin does not correlate with increased delafloxacin MICs (Figure 5).**

Table 2. *in vitro* Activity Against Contemporary Clinical Isolates from UAB (Study 2)

Compound	MIC Range	MIC 50	MIC 90
Penicillin	≤0.25 - 32	0.5	4
Spectinomycin	Susceptible		
Cefixime	≤0.015 - 1	≤0.015	0.125
Ceftriaxone	≤0.008 - 1	≤0.008	0.06
Ciprofloxacin	≤0.015 - 16	≤0.015	8
Delafloxacin	≤0.0005 – 0.06	0.001	0.06
Azithromycin	0.03 - 32	0.25	4

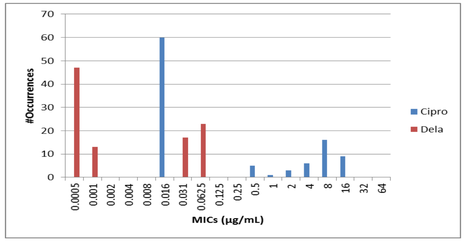


Figure 4. MIC range and distribution for delafloxacin and ciprofloxacin in Study 2.

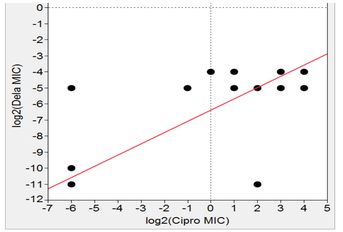


Figure 5. Delafloxacin activity, in log-2 scale, as a function of ciprofloxacin activity in Study 2.

Conclusions

The results of both studies show that delafloxacin is highly active against recent clinical isolates of ciprofloxacin- and multidrug-resistant *N. gonorrhoeae*, with superior *in vitro* activity compared with ciprofloxacin and the other antimicrobial agents tested. Notably, the MICs of delafloxacin in these studies are similar to those observed in two earlier studies, in 2007 and 2010 (10), where – in collections with high ciprofloxacin resistance (33 – 73%) – the MIC_{90}s were 0.125 µg/mL. These data are supportive of the ongoing development program to evaluate the clinical efficacy of delafloxacin to treat gonococcal infections, including from multi-drug resistant strains.

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