KPI-10, a Novel Fluoroquinolone (FQ) Tested Against *Neisseria gonorrhoeae* including Ciprofloxacin Non-Susceptible (CIP-NS) and Penicillin Non-Susceptible (PEN-NS) Strains

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Amended Abstract

Background: KPI-10 (WQ-3813) is a novel FQ agent having potent broad-spectrum activity against Gram-positive and -negative bacteria including select FQ-resistant (FQR) strains. In this study, KPI-10 and comparator agents were evaluated against a collection of *Neisseria gonorrhoeae* (NG) including CIP-NS and PEN-NS strains.

Methods: 99 NG isolates were selected from a well characterized collection of strains, mostly from the USA, but including isolates from Japan and the Netherlands, with the following antimicrobial resistance phenotypes: ciprofloxacin-susceptible (13), ciprofloxacin-intermediate (31), ciprofloxacin-resistant (55). MIC values were determined using the reference CLSI agar dilution method for NG on GC agar base media with the defined supplement.

Results: Ceftriaxone was the most potent agent overall with a MIC_{90} value of 0.12 µg/ml. KPI-10 was the most potent FQ with a MIC_{90} value of 0.12 µg/ml. KPI-10 activity was >16-fold greater than ciprofloxacin (MIC_{90}, >2 µg/ml) and >32-fold greater than levofloxacin (MIC_{90}, >4 µg/ml). The MIC_{90} value for KPI-10 for the ciprofloxacin-susceptible subset of isolates was 0.015 µg/ml; 0.06 µg/ml for the ciprofloxacin-intermediate strains, and 0.12 µg/ml for the ciprofloxacin-resistant subset. Against a PEN resistant subset, and a ciprofloxacin- and PEN-resistant subset, the MIC_{90} values for KPI-10 were 0.12 and 0.25 µg/ml, respectively. This was ≥32-fold more potent than levofloxacin and ≥16-fold more potent than penicillin. KPI-10 was equally as potent as ceftriaxone against PEN-resistant NG, however, it was eight-fold less potent against the ciprofloxacin- and PEN-resistant NG.

Conclusions: KPI-10 was shown to be the most potent FQ against NG with activity against CIP-NS and PEN-NS isolates. Its potent activity including activity against resistant subsets suggests that KPI-10 may be a candidate for evaluation in the treatment of gonococcal disease.

Introduction

Resistance development in *Neisseria gonorrhoeae* to several antimicrobial class agents used for empiric therapy of gonococcal infections, including fluoroquinolones (ciprofloxacin), tetracyclines and azithromycin among the macrolides has emerged. Recently, the World Health Organization has listed *N. gonorrhoeae* as a potential "untreatable" pathogen after the emergence of a multiple-drug resistant *N. gonorrhoeae* strain identified in Japan. Fluoroquinolones have been used in the treatment of a variety of human infections and are one of the most widely applied broad-spectrum antimicrobial classes. Fluoroquinolone-resistant *N. gonorrhoeae* strains are now widely disseminated throughout the US and the world and due to significantly reduced susceptibility, fluoroquinolones are no longer recommended as first-line therapy in the US, United Kingdom, Canada, Asia, or Australia for the treatment of gonorrhea-associated sexually transmitted diseases. Fluoroquinolone resistance in *N. gonorrhoeae* occurs due to DNA gyrase and topoisomerase IV mutations in the quinolone resistance determining region (QRDR), decreased uptake, or efflux of these agents.

KPI-10 (formerly WQ-3813, the maelic acid salt of WQ-3810) is a novel broad spectrum fluoroquinolone containing a 6-amino-3,5-difluoropyridine at the 1-position and 3-isopropylaminoazetidine at the 7-position which has potent activity against a variety of Gram-positive and -negative bacteria including select drug-resistant strains. In this study, KPI-10 and comparator fluoroquinolones were evaluated against a global collection of *N. gonorrhoeae*, including drug-resistant *N. gonorrhoeae.*

Materials & Methods

N. gonorrhoeae (99 isolates) were selected from a well characterized global collection of strains with the following antimicrobial resistance phenotypes: 1) ciprofloxacin-susceptible (n=13), 2) ciprofloxacin-intermediate (n=31), and 3) ciprofloxacin-resistant (n=55). These isolates also were analyzed/grouped based on their penicillin-susceptibility.

KPI-10 antimicrobial powder was provided by Kalidex. KPI-10 antimicrobial powder was dissolved in distilled water to make a stock solution for testing of 1280 µg/ml. Antimicrobials tested were KPI-10, ciprofloxacin, penicillin, levofloxacin, ceftriaxone and tetracycline. MIC values were determined using the reference agar dilution for *N. gonorrhoeae* as described in M07-A9 [2012]. Agar dilution (AD) plates (GC agar base with 1% defined supplement) for *N. gonorrhoeae* testing were produced by JMI Laboratories, North Liberty, Iowa, USA. GC agar base was provided by Oxoid (Basingstoke, Hampshire, England), Quality control (QC) ranges for *N. gonorrhoeae* ATCC 49226 and interpretive criteria for the comparator compounds were as published in CLSI M100-S22 (2012).

Results

- The MIC distributions for KPI-10 and comparator fluoroquinolones for *N. gonorrhoeae* are presented in Table 1. KPI-10 was the most potent of the fluoroquinolone agents with a MIC_{90} value of 0.12 µg/ml. This was >16-fold greater activity than ciprofloxacin and >32-fold greater activity than levofloxacin, which had MIC_{90} values of >2 and >4 µg/ml, respectively.
- Against this collection of 99 isolates containing ciprofloxacin-susceptible, -intermediate, and -resistant isolates as well as penicillin-resistant isolates (ciprofloxacin and penicillin MIC_{90} values of >2 µg/ml), KPI-10 and ceftriaxone were the most potent agents tested with MIC_{90} values of 0.12 µg/ml (Table 2).
- The MIC_{90} value for KPI-10 for the ciprofloxacin-susceptible subset of isolates was 0.015 µg/ml; 0.06 µg/ml for the ciprofloxacin-intermediate subset, and 0.12 µg/ml for the ciprofloxacin-resistant subset.
- Against the penicillin-resistant subset and the ciprofloxacin-resistant and penicillin-resistant subset the MIC_{90} values for KPI-10 were 0.12 and 0.25 µg/ml, respectively. This was at least ≥32-fold more potent than levofloxacin and at least ≥16-fold more potent than penicillin. KPI-10 was equally as potent as ceftriaxone against the penicillin-resistant subset; however, it was eight-fold less potent against the ciprofloxacin-resistant and penicillin-resistant subset.

Table 1. MIC frequency distribution and cumulative percent inhibited for KPI-10 and comparator fluoroquinolone antimicrobial agents tested against *N. gonorrhoeae*.

Organism/ antimicrobial agent (no. tested)	No. (cumulative %) of isolates inhibited at antimicrobial MIC (µg/ml):												MIC_{50}	MIC_{90}
	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	≥4		
N. gonorrhoeae (all strains, 99)														
KPI-10	3 (3.0)	7 (10.1)	1 (11.1)	11 (22.2)	16 (38.4)	13 (51.5)	43 (95.0)	5 (100.0)	-[a]	-	-	NT[b]	0.06	0.12
Ciprofloxacin	4 (4.0)	1 (5.1)	5 (10.1)	0 (10.1)	0 (10.1)	3 (13.1)	12 (25.3)	19 (44.4)	0 (44.4)	5 (49.5)	23 (72.7)	27 (100.0)	2	>2
Levofloxacin	NT	NT	5 (5.5)	4 (9.1)	1 (10.1)	1 (11.1)	1 (12.1)	13 (25.3)	20 (45.5)	3 (48.5)	8 (56.6)	43 (100.0)	2	>4
N. gonorrhoeae (Ciprofloxacin-susceptible, 13)														
KPI-10	3 (23.1)	7 (76.9)	0 (76.9)	2 (92.3)	1 (100.0)	-	-	-	-	-	-	NT	0.004	0.015
Ciprofloxacin	4 (30.8)	1 (38.5)	5 (76.9)	0 (76.9)	0 (76.9)	3 (100.0)	-	-	-	-	-	NT	0.008	0.06
Levofloxacin	NT	NT	5 (38.5)	4 (69.2)	1 (76.9)	0 (76.9)	1 (84.6)	2 (100.0)	-	-	-	-	0.015	0.25
N. gonorrhoeae (Ciprofloxacin-intermediate, 31)														
KPI-10	-	-	1 (3.2)	8 (29.0)	12 (67.7)	10 (100.0)	-	-	-	-	-	NT	0.03	0.06
Ciprofloxacin	-	-	-	-	-	-	12 (38.7)	19 (100.0)	-	-	-	NT	0.25	0.25
Levofloxacin	NT	NT	-	-	-	-	-	11 (35.5)	20 (100.0)	-	-	-	0.5	0.5
N. gonorrhoeae (Ciprofloxacin-resistant, 55)														
KPI-10	-	-	-	1 (1.8)	3 (7.3)	3 (12.7)	43 (90.9)	5 (100.0)	-	-	-	NT	0.12	0.12
Ciprofloxacin	-	-	-	-	-	-	-	-	-	5 (9.1)	23 (50.9)[b]	27 (100.0)	2	>2
Levofloxacin	NT	NT	-	-	-	1 (1.8)	0 (1.8)	0 (1.8)	0 (1.8)	3 (7.3)	8 (21.8)	43 (100.0)	4	>4

a. A dash indicates that no values were observed at that MIC value.
b. NT = dilution was not tested.

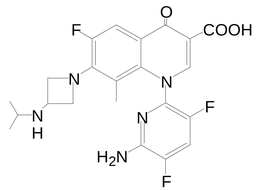
Table 2. Activity of KPI-10 and comparator antimicrobial agents when tested against isolates of *N. gonorrhoeae*.

Organism/ antimicrobial agent (no. tested)	MIC (µg/ml)			CLSI[a]	EUCAST[a]
	MIC_{50}	MIC_{90}	Range	%S / %R	%S / %R
Neisseria gonorrhoeae (99)					
KPI-10	0.06	0.12	0.002 – 0.25	- / -	- / -
Ciprofloxacin	2	>2	0.002 – >2	13.1 / 55.6	10.1 / 86.9
Levofloxacin	2	>4	≤0.008 – >4	- / -	- / -
Penicillin	1	>2	0.25 – >2	0.0 / 49.5	0.0 / 49.5
Ceftriaxone	0.015	0.12	≤0.008 – 0.12	100.0 / -	100.0 / 0.0
Tetracycline	1	2	0.12 – >4	3.0 / 44.4	21.2 / 44.5
Neisseria gonorrhoeae (ciprofloxacin-susceptible, 13)					
KPI-10	0.004	0.015	0.002 – 0.03	- / -	- / -
Ciprofloxacin	0.008	0.06	0.002 – 0.06	100.0 / 0.0	76.9 / 0.0
Levofloxacin	0.015	0.25	≤0.008 – 0.25	- / -	- / -
Penicillin	1	>2	0.25 – >2	0.0 / 46.2	0.0 / 46.2
Ceftriaxone	0.015	0.03	≤0.008 – 0.06	100.0 / -	100.0 / 0.0
Tetracycline	2	>4	0.25 – >4	7.7 / 53.8	38.5 / 53.8
Neisseria gonorrhoeae (ciprofloxacin-intermediate, 31)					
KPI-10	0.03	0.06	0.008 – 0.06	- / -	- / -
Ciprofloxacin	0.25	0.25	0.12 – 0.25	0.0 / 0.0	0.0 / 100.0
Levofloxacin	0.5	0.5	0.25 – 0.5	- / -	- / -
Penicillin	2	>2	1 – >2	0.0 / 67.7	0.0 / 67.7
Ceftriaxone	0.03	0.12	≤0.008 – 0.12	100.0 / -	100.0 / 0.0
Tetracycline	1	2	1 – 2	0.0 / 38.7	0.0 / 38.7

Organism/ antimicrobial agent (no. tested)	MIC (µg/ml)			CLSI[a]	EUCAST[a]
	MIC_{50}	MIC_{90}	Range	%S / %R	%S / %R
Neisseria gonorrhoeae (ciprofloxacin-resistant, 55)					
KPI-10	0.12	0.12	0.015 – 0.25	- / -	- / -
Ciprofloxacin	2	>2	1 – >2	0.0 / 100.0	0.0 / 100.0
Levofloxacin	4	>4	0.06 – >4	- / -	- / -
Penicillin	1	>2	0.25 – >2	0.0 / 40.0	0.0 / 40.0
Ceftriaxone	0.015	0.03	≤0.008 – 0.06	100.0 / -	100.0 / 0.0
Tetracycline	1	4	0.12 – >4	3.6 / 45.5	29.1 / 45.5
Neisseria gonorrhoeae (penicillin-resistant, 49)					
KPI-10	0.06	0.12	0.002 – 0.25	- / -	- / -
Ciprofloxacin	0.25	>2	0.002 – >2	12.2 / 44.9	10.2 / 87.8
Penicillin	2	>2	2 – >2	0.0 / 100.0	0.0 / 100.0
Levofloxacin	0.5	>4	≤0.008 – >4	- / -	- / -
Ceftriaxone	0.03	0.12	≤0.008 – 0.12	100.0 / -	100.0 / 0.0
Tetracycline	2	4	0.25 – >4	4.1 / 59.2	16.3 / 59.2
Neisseria gonorrhoeae (ciprofloxacin-resistant/penicillin-resistant, 22)					
KPI-10	0.12	0.25	0.015 – 0.25	- / -	- / -
Ciprofloxacin	>2	>2	1 – >2	0.0 / 100.0	0.0 / 100.0
Penicillin	2	>2	2 – >2	0.0 / 100.0	0.0 / 100.0
Levofloxacin	>4	>4	1 – >4	- / -	- / -
Ceftriaxone	0.03	0.03	≤0.008 – 0.06	100.0 / -	100.0 / 0.0
Tetracycline	2	4	0.25 – >4	4.5 / 68.2	22.7 / 68.2

a. Criteria as published by the CLSI [2012] and EUCAST [2012].

Figure 1. Chemical structure of KPI-10 (WQ-3810)



Conclusions

- KPI-10 was the most potent of the fluoroquinolones against *N. gonorrhoeae* with activity against ciprofloxacin and penicillin-resistant isolates (overall $MIC_{50/90}$ values of 0.06/0.12 µg/ml).
- KPI-10 was >16-fold more active than ciprofloxacin and >32-fold more active than levofloxacin which had MIC_{90} values of >2 and >4 µg/ml, respectively.
- The potent activity of KPI-10, including activity against resistant subsets, suggests it may be a candidate for evaluation in the treatment of gonococcal disease, including sexually transmitted diseases caused by fluoroquinolone-resistant *N. gonorrhoeae.*

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References

1. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition*. Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2012). *M100-S22. Performance standards for antimicrobial susceptibility testing: 22nd informational supplement*. Wayne, PA: CLSI.
3. Dan M (2004). The use of fluoroquinolones in gonorrhoea: the increasing problem of resistance. *Expert Opin Pharmacother* 5: 829-854.
4. Kazamori D, Suzuki H, Amago T, Itoh K, Yazaki A (2009). In vitro activity of WQ-3813 against nosocomial pathogens. Abstr. F1-1984. *49th ICAAC, September 12-15, 2009*, San Francisco, CA, USA.
5. Lee H, Hong SG, Soe Y, Yong D, Jeong SH, Lee K, Chong Y (2011). Trends in antimicrobial resistance of *Neisseria gonorrhoeae* isolated from Korean patients from 2000 to 2006. *Sex Transm Dis* 38: 1082-1086.
6. Martin I, Jayaraman G, Wong T, Liu G, Gilmour M (2011). Trends in antimicrobial resistance in *Neisseria gonorrhoeae* isolated in Canada: 2000-2009. *Sex Transm Dis* 38: 892-898.
7. Ohnishi M, Golparian D, Shimuta K, Saika T, Hoshina S, Iwasaku K, Nakayama S, Kitawaki J, Unemo M (2011). Is *Neisseria gonorrhoeae* initiating a future era of untreatable gonorrhea?: Detailed characterization of the first strain with high-level resistance to ceftriaxone. *Antimicrob Agents Chemother* 55: 3538-3545.
8. Suzuki H, Hayashi N, Amago T, Takenaka H, Amano H, Itoh K, Kuramoto Y, Yazaki A (2008). WQ-3810, a next generation respiratory fluoroquinolone with outstanding activity against QRSP. Abstr. F1-2051. *48th ICAAC, October 25-28, 2008*, Washington DC, USA.
9. Tapsall JW (2006). What management is there for gonorrhea in the postquinolone era? *Sex Transm Dis* 33: 8-10.
10. Uehara AA, Amorin EL, Ferreira Mde F, Andrade CF, Clementino MB, de Filippis I, Neves FP, Pinto Tde C, Teixeira LM, Giambiagi-Demarval M, Fracalanzza SE (2011). Molecular characterization of quinolone-resistant *Neisseria gonorrhoeae* isolates from Brazil. *J Clin Microbiol* 49: 4208-4212.