Activity of JNJ-Q2, a Novel Fluoroquinolone, Tested against *Neisseria gonorrhoeae*, Including Ciprofloxacin-Resistant Strains

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AMENDED ABSTRACT

Background: JNJ-Q2, a novel 4-fluoro-quinolone (FQ), has been shown to be effective in a Phase II study for ABSSSI. Here we tested its' *in vitro* activity against *N. gonorrhoeae* (GC) isolates, including genetically defined CIP-resistant (R) strains.

Methods: Among 75 GC isolates, approximately one-third of strains were susceptible (S) or intermediate (I) or R to CIP using CLSI breakpoint criteria (69.3% R using EUCAST criteria). Genetically defined CIP-R strains (31) had mutations in the quinolone resistance determining region (QRDR) including *gyr*A (positions 91 and/or 95) and *par*C (positions 86, 87, 88 or 91). MIC values were determined using CLSI agar dilution methods on GC agar with defined growth supplement. Comparators included CIP, penicillin (PEN), ceftriaxone (CRO), tetracycline (TET) and azithromycin (AZI).

Results: All GC were inhibited by ≤0.25 µg/ml of JNJ-Q2 with a range of 0.004 – 0.25 µg/ml ($MIC_{50/90}$, 0.03/0.25 µg/ml). This activity was eight-fold (MIC_{50}) and 32-fold greater (MIC_{90}) compared to CIP. Only 3.0% of strains were PEN-S, 6.1% were TET-S and using EUCAST criteria, 13.6% were R to AZI. All strains were CRO-S, the most potent agent (MIC_{90}, 0.06 µg/ml) followed by JNJ-Q2 (MIC_{90}, 0.25 µg/ml). JNJ-Q2 was eight- to 16-fold and 32- to 64-fold more active compared to CIP among CIP-I and CIP-R strains, respectively.

Conclusions: JNJ-Q2 is a very potent FQ when tested against contemporary multidrug-R (MDR) GC. Comparative activity shows greater activity against emerging MDR strains. The data suggests JNJ-Q2 has the potential to be a valuable new agent for GC, particularly in light of concerns with emerging antibiotic resistance.

INTRODUCTION

Neisseria gonorrhoeae is the causative agent of gonorrhea, a sexually transmitted disease of worldwide importance. Historically, gonorrhea was effectively treated with penicillin but since first being reported in 1976, plasmid-mediated penicillinase-producing *N. gonorrhoeae* (PPNG) have spread worldwide, hence limiting the empiric use of penicillin to certain geographical regions of known low-prevalence of PPNG.

Over the past few decades, resistance development has occurred for agents that have been used as empiric therapy (ciprofloxacin, tetracyclines and azithromycin) necessitating the need for third-generation cephalosporins (ceftriaxone and cefixime) as agents for empiric and targeted treatment. In analyzing data from the United States from 2000 to 2010, the Gonococcal Isolate Surveillance Project recently reported a rapid increase in the percentage of gonorrhea isolates from 2009 and 2010 with cefixime MIC results at ≥0.25 µg/mL and ceftriaxone MIC values of ≥0.125 µg/mL. Of greatest concern, a high level (MIC, 2 µg/ml) ceftriaxone-resistant strain with a new *penA* mosaic structure was recently reported in a female sex worker in Japan. Clearly, new treatment options for gonorrhea are urgently needed.

JNJ-Q2 is a novel fluorinated 4-quinolone with demonstrated potent activity against Gram-positive (including MRSA and multidrug-resistant pneumococci) and Gram-negative pathogens. Also JNJ-Q2 has been shown to have balanced potency against both DNA gyrase and topoisomerase IV targets. JNJ-Q2 was shown to be effective in a Phase II study for acute bacterial skin and skin structure infection. In this preliminary investigation to assess the potential for JNJ-Q2 to treat gonorrhea, JNJ-Q2 was evaluated against a collection of 75 *N. gonorrhoeae* strains, including 31 genetically defined ciprofloxacin-resistant strains.

MATERIALS AND METHODS

Susceptibility testing. MIC values for JNJ-Q2 and comparator agents were determined using the reference Clinical and Laboratory Standards Institute (CLSI) agar dilution method as described in M07-A8 [2009]. Agar dilution plates were produced by JMI Laboratories (North Liberty, Iowa, USA) and consisted of GC agar with 1% defined growth supplement according to the CLSI testing conditions M100-S21 [2011].

Quality Control (QC) using the American Type Culture Collection (ATCC) *N. gonorrhoeae* ATCC 49226 strain was performed during each testing event, at least in duplicate, with five total replicate values reported and all values were within specified MIC limits. Interpretive criteria for the direct comparator compound (ciprofloxacin) and additional compounds were as published in CLSI M100-S21 [2011]. Interpretive criteria defined by EUCAST were also utilized, when applicable [EUCAST, 2011].

Bacterial isolates. A collection of 75 *N. gonorrhoeae* strains, including 31 genetically defined ciprofloxacin-nonsuceptible strains, were evaluated. These strains were clinical isolates from the JMI Laboratories bacterial collection with identifications confirmed by at least two independent laboratories. Almost one-third of the strains were either susceptible (30.7%) or resistant (33.3%) to ciprofloxacin, with the remainder (36.0%) having an intermediate-level MIC values to this fluoroquinolone using CLSI breakpoint criteria (69.3% resistant using EUCAST criteria). Multidrug-resistance (MDR) was defined as resistance to two or more antimicrobial agents from the following list: ciprofloxacin, penicillin, ceftriaxone, tetracycline, and azithromycin.

All genetically defined ciprofloxacin-resistant strains had documented mutations in the quinolone resistance determining region (QRDR). These 31 isolates were shown to have amino acid *gyr*A mutations at positions 91 and/or 95 and eleven of the isolates also had additional amino acid *par*C mutations at positions 86, 87, 88 or 91 in the QRDR.

RESULTS

- Combined, all 75 clinical isolates of *N. gonorrhoeae* were inhibited by ≤0.25 µg/ml of JNJ-Q2 (Tables 1 and 2). The range of JNJ-Q2 values was only 0.004 – 0.25 µg/ml with MIC_{50} and MIC_{90} values of 0.03 µg/ml and 0.25 µg/ml, respectively. This activity was eight-fold greater using MIC_{50} values and 32-fold greater using MIC_{90} values when compared to ciprofloxacin. The MIC values for ciprofloxacin were elevated to as high as 16 µg/ml for seven strains and JNJ-Q2 MIC values were only 0.12 – 0.25 µg/ml for these most resistant strains.
- For the overall collection, only 3.0% of strains were susceptible to penicillin and 6.1% were susceptible to tetracycline using the CLSI breakpoint criteria (Table 2). Applying EUCAST breakpoint criteria, 13.6% of isolates would be considered resistant to azithromycin. However, all strains were susceptible to ceftriaxone, which was the most potent antimicrobial agent tested (MIC_{90}, 0.06 µg/ml) followed by JNJ-Q2 (MIC_{90}, 0.25 µg/ml).
- The MIC_{90} values for JNJ-Q2 were 0.015 µg/ml, 0.03 µg/ml and 0.25 µg/ml for ciprofloxacin-susceptible, -intermediate and -resistant (Table 2) strains, respectively.
- When stratified by ciprofloxacin susceptibility, it was observed that JNJ-Q2 had similar activity (MIC_{90}, 0.015 µg/ml) to ciprofloxacin (MIC_{90}, 0.015 µg/ml) against susceptible isolates. However, JNJ-Q2 was eight- to 16-fold more active compared to the ciprofloxacin-intermediate collection of isolates and 32- to 64-fold more active compared to the ciprofloxacin-resistant collection (Table 2).
- When analyzed by the QRDR mutations present, JNJ-Q2 MIC values did not increase in strains with two mutations (one on each of GyrA and ParC) compared to strains with single mutations in GyrA (Table 3). In contrast, ciprofloxacin MIC values increased up to eight-fold between strains with double compared to single mutations. In strains with three mutations (two in GyrA and one in ParC) ciprofloxacin MIC values increased up to 16 µg/ml while JNJ-Q2 values four- to eight-fold but no higher than 0.25 µg/ml (Table 3).
- JNJ-Q2 was also very active ($MIC_{50/90}$, 0.03/0.25 µg/ml) against thirty MDR *N. gonorrhoea* strains with 96.7% demonstrating ciprofloxacin non-susceptibility (53.3% resistant; Table 2).

Table 1. JNJ-Q2 MIC frequency distributions when tested against 75 isolates of *N. gonorrhoeae*.

	No. (cum. %) of isolates inhibited at JNJ-463 MIC (µg/ml)						
Species (no. tested)	0.004	0.008	0.015	0.03	0.06	0.12	0.25
N. gonorrhoeae (75)	1 (1.3)	7 (10.7)	15 (30.7)	29 (69.3)	1 (70.7)	14 (89.3)	8 (100.0)
Ciprofloxacin-susceptible (23)	1 (4.2)	7 (33.3)	15 (100.0)	-	-	-	-
Ciprofloxacin-intermediate (27)	0 (0.0)	0 (0.0)	0 (0.0)	27 (100.0)	-	-	-
Ciprofloxacin-resistant (25)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.0)	1 (12.0)	14 (68.0)	8 (100.0)

Table 2. Antimicrobial activity of JNJ-Q2 and comparator antimicrobials against *N. gonorrhoeae*.

Organism (no. tested)/ Antimicrobial agent[a]	MIC in µg/ml MIC_{50}	MIC_{90}	Range	CLSI[b] %S[c] / %R[d]	EUCAST[b] %S / %R
N. gonorrhoeae					
All isolates (75)					
JNJ-Q2	0.03	0.25	0.004 – 0.25	- / -	- / -
Ciprofloxacin	0.25	8	0.002 – 16	30.7 / 33.3	29.3 / 69.3
Penicillin[b]	2	>4	0.03 – >4	3.0 / 59.1	3.0 / 59.1
Ceftriaxone[b]	0.015	0.06	≤0.008 – 0.12	100.0 / -	100.0 / 0.0
Tetracycline[b]	1	2	0.12 – >4	6.1 / 40.9	28.8 / 40.9
Azithromycin[b]	0.25	1	0.06 – 2	- / -	56.1 / 13.6
Ciprofloxacin-S[c] (23)					
JNJ463	0.015	0.015	0.004 – 0.015	- / -	- / -
Ciprofloxacin	0.004	0.015	0.002 – 0.06	100.0 / 0.0	100.0 / 0.0
Penicillin	0.5	4	0.03 – >4	7.1 / 14.3	7.1 / 14.3
Ceftriaxone	≤0.008	0.03	≤0.008 – 0.06	100.0 / -	100.0 / 0.0
Tetracycline	0.5	1	0.12 – 2	14.3 / 7.1	64.3 /7.1
Azithromycin	0.12	0.25	0.06 – 1	- / -	92.9 /7.1
Ciprofloxacin-I[d] (27)					
JNJ-Q2	0.03	0.03	0.03 – 0.03	- / -	- / -
Ciprofloxacin	0.25	0.5	0.12 – 0.5	0.0 / 0.0	0.0 / 100.0
Penicillin	2	>4	0.03 – >4	3.7 / 88.9	3.7 / 88.9
Ceftriaxone	0.03	0.06	≤0.008 – 0.12	100.0 / -	100.0 / 0.0
Tetracycline	1	2	0.5 – >4	0.0 / 48.1	11.1 / 48.1
Azithromycin	0.25	1	0.06 – 1	- / -	55.6 / 14.8
Ciprofloxacin-R[e] (25)					
JNJ-Q2	0.12	0.25	0.03 – 0.25	- / -	- / -
Ciprofloxacin	4	16	1 – 16	0.0 / 100.0	0.0 / 100.0
Penicillin	1	>4	1 – >4	0.0 / 52.0	0.0 / 52.0
Ceftriaxone	0.015	0.03	≤0.008 – 0.06	100.0 / -	100.0 / 0.0
Tetracycline	2	4	0.12 – >4	8.0 / 52.0	28.0 / 52.0
Azithromycin	0.5	1	0.12 – 1	- / -	36.0 / 16.0
Multidrug-resistant[f,g] (30)					
JNJ-Q2	0.03	0.25	0.015 – 0.25	- / -	- / -
Ciprofloxacin	1	16	1 – 16	3.3 / 53.3	[h]
Penicillin	4	>4	1 - >4	0.0 / 90.0	[h]
Ceftriaxone	0.03	0.06	≤0.008 – 0.06	100.0 / -	[h]
Tetracycline	2	4	0.25 – >4	3.3 / 90.0	[h]
Azithromycin	0.5	1	0.06 – 2	[h]	33.3 / 36.7

a. Criteria as published by the CLSI [2011] and EUCAST [2011].
b. Includes only 66 isolates that had data available for comparison.
c. S = susceptible.
d. I = intermediate.
e. R = resistant.
f. Multidrug-resistance defined as resistance to two or more antimicrobial agents from the following list: ciprofloxacin, penicillin, ceftriaxone, tetracycline, and azithromycin.
g. CLSI breakpoints alone were used for this subset except for azithromycin for which EUCAST breakpoints were used (there are no CLSI breakpoints for azithromycin against *N. gonorrhoeae*).
h. Data not shown as these interpretations were not used to determine MDR status.

Table 3. Range of antimicrobial activity of JNJ-Q2 and ciprofloxacin against 31 strains of *N. gonorrhoeae* with defined fluoroquinolone resistance mechanisms.

		MIC range (µg/ml)	
QRDR mutations	N	JNJ-Q2	Ciprofloxacin
GyrA (S91F)	14	0.015 - 0.06	0.06 - 1
GyrA (S91Y)	2	0.03	0.12
GyrA (D95N)	4	0.03	0.12 - 0.25
GyrA (S91F), ParC (D86N)	6	0.03	0.25 -0.5
GyrA (S91F), ParC (S87I)	2	0.03	1
GyrA (S91F), GyrA (D95N), ParC (S88P)	2	0.12	2
GyrA (S91F), GyrA (D95G), ParC (E91G)	1	0.25	16

CONCLUSIONS

- JNJ-Q2 is a very active new oral fluoroquinolone agent when tested *in vitro* against *N. gonorrhoeae*. Comparison to an in-class agent shows greater activity and potential efficacy against strains resistant to other fluoroquinolones and MDR strains by CLSI and EUCAST susceptibility criteria.
- JNJ-Q2 would be a likely fluoroquinolone candidate for the evaluation of treating infections that are caused by multidrug-resistant *N. gonorrhoeae.* This agent should be tested further against ceftriaxone non-susceptible strains and those with high MIC values to azithromycin.

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