Activity of JNJ-Q2 and Comparators against Genetically Defined MRSA Clones

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AMENDED ABSTRACT

Background: JNJ-Q2 is a broad-spectrum bactericidal fluoroquinolone (FQ) with potent activity against Gram-positive pathogens including methicillin-resistant (MR) *S. aureus* (SA), Gram-negative pathogens, atypical respiratory pathogens, and anaerobic pathogens and is in clinical development for the treatment of acute bacterial skin and skin structure infection (ABSSSI) and community-acquired bacterial pneumonia. In this study, the in vitro activity of JNJ-Q2 and other FQ agents was evaluated against a panel of genetically defined strains representative of global MRSA clones.

Methods: A collection of 111 genetically defined SA strains (predominantly MRSA [103/111, 92.8%]) representative of the major circulating global clones were evaluated. Along with the groups specified in the table (below), USA PFGE types 100 to 1100 (not 900), SCCmec types I to IV (and IV-a), agr types I to IV, and PVL-positive strains were all represented. Isolates were tested against JNJ-Q2, moxifloxacin (MOX), levofloxacin (LEV), and other comparator agents per the CLSI broth microdilution method (M07-A8; M100-S21).

Results: Overall, JNJ-Q2 inhibited all 111 major circulating *S. aureus* clones at ≤1 µg/ml. MOX and LEV demonstrated high levels of resistance (R) to these strains (69.4 and 73.9%, respectively). JNJ-Q2 inhibited all ten VRSA strains at a MIC of ≤1 µg/ml and all ten were R to MOX and LEV. JNJ-Q2 was also very active against strains R to comparator agents (n strains): daptomycin (7), linezolid (5), tetracycline (32), trimethoprim-sulfamethoxazole (13), erythromycin (86) and clindamycin (41).

Conclusion: JNJ-Q2 retained activity against these sub-sets of challenging strains regardless of antimicrobial resistance phenotype, ST or PFGE clonal types, PVL positivity, and agr or SCCmec type. These JNJ-Q2 in vitro results against major global MRSA clones are very promising and support further clinical development of this new FQ for treatment of ABSSSI.

Abstract Table

	JNJ-Q2		Levofloxacin		Moxifloxacin	
Group or clone (n)	$MIC_{50/90}$	Range	$MIC_{50/90}$	Range	$MIC_{50/90}$	Range
All strains (111)	0.12/0.25	≤0.06 – 1	8/32	0.12 – >128	2/8	≤0.06 – >128
ST239[a] (12)	0.25/0.25	0.12-0.25	16/32	4-32	4/8	1->128
ST8 USA300 (23)	≤0.06/0.12	≤0.06-1	0.25/4	0.12-4	1/2	≤0.06-2
ST22[b] (15)	0.12/0.25	≤0.06-0.5	8/32	0.12-32	2/8	≤0.06-8
ST5[c] (5)	0.12/-	0.12-1	8/-	8-16	2/-	2-4
ST5 USA100 (6)	0.25/-	0.25-1	32/-	32->128	8/-	4-32
VRSA (10)	0.25/0.25	0.12-1	16/64	16->128	8/8	4-32

a. ST239 Hungarian/Brazilian.
b. ST22 EMRSA-15.
c. ST5 Cordobes/Chilian.

INTRODUCTION

JNJ-Q2 is a novel fluorinated 4-quinolone with potent activity against Gram-positive (including MRSA) and Gram-negative pathogens that has been demonstrated to have balanced potency against DNA gyrase and topoisomerase IV. JNJ-Q2 is in clinical development for the treatment of acute bacterial skin and skin structure infections (ABSSSI's) and community-acquired bacterial pneumonia (CABP).

Previously, JNJ-Q2 was shown to be active against 511 selected *S. aureus* isolates (including 308 fluoroquinolone-resistant MRSA) collected between 2008 to 2009, with levofloxacin/JNJ-Q2 MIC_{50} values, MIC_{90} values, and MIC ranges of 8/0.12, >16/0.5 and 0.12 - >16/≤0.008 - 4 µg/ml, respectively.

The objective of this study was to determine the in vitro activity of JNJ-Q2 tested against a panel of genetically defined strains representative of global MRSA clones.

MATERIALS AND METHODS

Susceptibility testing. MIC values were determined using the reference CLSI broth microdilution method as described in M07-A8 [2009]. For the four tested fluoroquinolones: 96-well, frozen-form panels were produced by JMI Laboratories (North Liberty, Iowa, USA) consisting of one media type e.g., cation-adjusted Mueller-Hinton broth. For the comparator agents, the MIC data was obtained from the SENTRY Antimicrobial Surveillance Program database.

Quality Control (QC) was performed daily during the testing period and inoculum density was monitored by colony counts. QC ranges and interpretive criteria for the comparator compounds were as published in CLSI M100-S21 [2011]; tested QC strains included *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212. All QC values were within specified ranges.

Bacterial isolates. A collection of 111 genetically defined *S. aureus* strains (predominantly MRSA [103/111, 92.8%]) representative of the major circulating global clones were evaluated. These strains were from two sources: 1) clinical isolates from the JMI Laboratories bacterial strain collection; and 2) the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA), funded by the National Institute of Allergy and Infectious Diseases.

The following strains were tested:

- NARSA strains (30) included USA100 to 1100 clones (except for USA900), linezolid- and tigecycline-resistant strains, vancomycin-intermediate strains (VISA), strains positive for several toxin genes including Panton-Valentine Leukocidin (PVL).
- NARSA vancomycin-resistant *S. aureus* strains (VRSA, 10)
- ST239; Hungarian/Brazilian clone; SCC*mec* III (12 strains from 5 different countries).
- ST8; USA300; SCC*mec* IV; PVL-positive (23 clinical strains from the USA).
- ST22; EMRSA-15; European clone; SCC*mec* IV; PVL-negative (15 strains from 4 countries).
- ST5; Cordobes/Chilean clone; SCC*mec* I (5 strains from 4 countries)
- ST5; USA100 (6)
- Others (10)

RESULTS

- All strains combined (111 strains, Tables 1 and 2). The MIC distributions of JNJ-Q2 and comparison fluoroquinolones against all 111 strains are summarized in Table 1. JNJ-Q2 displayed very high potency ($MIC_{50/90}$, 0.12/0.25 µg/ml) against this panel of strains inhibiting all 111 strains at a MIC of ≤1 µg/ml. Using MIC_{50}, JNJ-Q2 demonstrated 16-, 64-, and 256-fold greater activity than moxifloxacin (69.4% resistant), levofloxacin (73.9% resistant) and ciprofloxacin (74.8% [75.7% by EUCAST criteria] resistant), respectively (Tables 1 and 2). Overall, 92.8% of strains were oxacillin resistant and resistance was high against erythromycin (77.5%), clindamycin (36.9 - 38.9%), tetracycline (28.8 – 30.6%) and trimethoprim/sulphamethoxazole (18.9%).
- NARSA strains (30 strains; Table 3). Against this reference panel of strains representing the major worldwide clones of MRSA, JNJ-Q2 inhibited all strains at ≤1 µg/ml and with a $MIC_{50/90}$ of 0.12/0.25 µg/ml that was 16-, 64- and 128-fold more active than moxifloxacin, levofloxacin and ciprofloxacin using MIC_{50} values, respectively.
- VRSA (10 strains; Table 3). JNJ-Q2 was the most active fluoroquinolone tested against VRSA ($MIC_{50/90}$, 0.25/0.25 µg/ml, Table 3). Significantly, all ten VRSA strains were resistant to moxifloxacin (MIC_{50}, 8 µg/ml), levofloxacin (MIC_{50}, 16 µg/ml) and ciprofloxacin (MIC_{50}, 64 µg/ml).
- ST239 Hungarian/Brazilian clone (12 strains; Table 3). This MRSA clone is prevalent in Europe and Latin America and increasing in prevalence in North America. JNJ-Q2 was also the most active fluoroquinolone tested against the ST239 Hungarian/Brazilian clone ($MIC_{50/90}$, 0.25/0.25 µg/ml, Table 3). The highest JNJ-Q2 MIC observed was only 0.25 µg/ml.
- ST8 USA300 (23 strains; Table 3). ST8 USA300 is the clone responsible for the majority of cases of community-acquired (CA-) MRSA in the USA and displayed the lowest fluoroquinolone MIC_{50} results among all groups analyzed (ranging from ≤0.06 [for JNJ-Q2] to 8 µg/ml [for ciprofloxacin]; Table 3).
- ST22 EMRSA-15 clone (15 strains; Tables 3). This clone is the predominant clone causing hospital infection in the United Kingdom. JNJ-Q2 was very active ($MIC_{50/90}$, 0.12/0.25 µg/ml, Table 3) against this clone and demonstrated much greater activities than the fluoroquinolone comparators (MIC_{50} values of 2 – 64 µg/ml).
- ST5 – USA100 (6 strains) and Cordobes/Chilian clone (5 strains; Table 3). Again, JNJ-Q2 was the most active fluoroquinolone agent against these clones with all strains being inhibited at a MIC ≤1 µg/ml compared to MIC values as high as >128 µg/ml for comparator fluoroquinolones.
- USA PFGE types (Table 3). JNJ-Q2 demonstrated high activity against the known USA PFGE types 100 to 1100 with all MIC values ≤1 µg/ml.
- PVL positive strains (8 strains; Table 3). All eight PVL-positive strains tested were inhibited by both JNJ-Q2 and moxifloxacin MIC values of ≤0.06 µg/ml.
- *agr* and SCC*mec* types (Table 3). JNJ-Q2 demonstrated high activity against all strains tested with known *agr* and SCCmec types. The highest JNJ-Q2 MIC_{90} for any subgroup of strains tested was 1 µg/ml against strains with SCC*mec* type I; corresponding MIC_{90} values for moxifloxacin, levofloxacin and ciprofloxacin were many-fold higher.
- Daptomycin-resistant strains. Seven strains were resistant to daptomycin and the JNJ-Q2 MIC values for these strains were ≤0.06 µg/ml (2 strains), 0.12 µg/ml (2 strains) and 0.25 µg/ml (3 strains).
- Vancomycin non-susceptible strains. Two strains were vancomycin-intermediate and the JNJ-Q2 MIC value for both strains was 0.25 µg/ml. Ten strains were VRSA and the JNJ-463 MIC values for these strains were 0.12 µg/ml (2 strains), 0.25 µg/ml (7 strains) and 1 µg/ml (1 strain).
- Linezolid-resistant strains. Five strains were resistant to linezolid and the JNJ-Q2 MIC values for these strains were ≤0.06 µg/ml (1 strain) and 0.25 µg/ml (4 strains).

Table 1. MIC frequency and cumulative percent inhibited distributions of JNJ-463 and comparator fluoroquinolone antimicrobial agents against all 111 *S. aureus*.

Antimicrobial agent	No. (cumulative %) of strains inhibited at antimicrobial MIC (µg/ml): ≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50}	MIC_{90}
JNJ-463	31 (27.9)	32 (56.8)	41 (93.7)	1 (94.6)	6 (100.0)									0.12	0.25
Ciprofloxacin	-[a]	1 (0.9)	14 (13.5)	11 (23.4)	1 (24.3)	1 (25.2)	0 (25.2)	7 (31.5)	14 (44.1)	13 (55.9)	24 (77.5)	15 (91.0)	10(100.0)	32	128
Levofloxacin	0 (0.0)	11 (9.9)	16 (24.3)	1 (25.2)	0 (25.2)	1 (26.1)	13 (37.8)	25 (60.4)	23 (81.1)	12 (91.9)	3 (94.6)	0(94.6)	6(100.0)	8	32
Moxifloxacin	25 (22.5)	3 (25.2)	0 (25.2)	0 (25.2)	6 (30.6)	37 (64.0)	19 (81.1)	14 (93.7)	0 (93.7)	6 (99.1)	0 (99.1)	0(99.1)	1(100.0)	2	8

a. Ciprofloxacin was not tested at this concentration and hence the isolate at 0.12 µg/ml is actually ≤0.12 µg/ml.

Table 2. Activity of JNJ-Q2 and comparator antimicrobial agents when tested against all 111 *S. aureus* strains.

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	CLSI[a] %S / %R	EUCAST[a] %S / %R	Antimicrobial agent	MIC_{50}	MIC_{90}	Range	CLSI[a] %S / %R	EUCAST[a] %S / %R
JNJ-Q2	0.12	0.25	≤0.06 – 1	- / -	- / -	Clindamycin	≤0.25	>2	≤0.25 – >2	61.3 / 36.9	59.5 / 38.7
Ciprofloxacin	32	128	≤0.12 – >128	24.3 / 74.8	24.3 / 75.7	Daptomycin	0.5	1	0.12 – >8	93.7 / -	93.7 / 6.3
Levofloxacin	8	32	0.12 – >128	25.2 / 73.9	25.2 / 73.9	Vancomycin	1	8	≤0.12 – >16	83.8 / 9.0	83.8 / 16.2
Moxifloxacin	2	8	≤0.06 – >128	25.2 / 69.4	25.2 / 69.4	Linezolid	1	2	0.5 – >8	95.5 / 4.5	95.5 / 4.5
Oxacillin	>2	>2	≤0.25 – >2	7.2 / 92.8	7.2 / 92.8	Tetracycline	≤2	>8	≤2 – >8	71.2 / 28.8	68.5 / 30.6
Erythromycin	>2	>2	≤0.25 – >2	22.5 / 77.5	22.5 / 77.5	Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5 – >2	81.1 / 18.9	81.1 / 18.9

a. Criteria as published by the CLSI (CLSI, 2011) and EUCAST (EUCAST, 2011).

Table 3. Activities of JNJ-Q2, ciprofloxacin, levofloxacin and moxifloxacin by reference group, clonal group, and other genetic markers.

	JNJ-Q2		Ciprofloxacin		Levofloxacin		Moxifloxacin	
Group or clone (n)	$MIC_{50/90}$	Range	$MIC_{50/90}$	Range	$MIC_{50/90}$	Range	$MIC_{50/90}$	Range
NARSA strains (30)	0.12/0.25	≤0.06-1	16/128	≤0.06->128	8/16	0.12->128	2/4	≤0.06-32
VRSA (10)	0.25/0.25	0.12-1	64/128	32->128	16/64	16->128	8/8	4-32
ST239 Hungarian/Brazilian (12)	0.25/0.25	0.12-0.25	64/128	8-128	16/32	4-32	4/8	1->128
ST8 USA300 (23)	≤0.06/0.12	≤0.06-1	8/16	0.25-32	0.25/4	0.12-4	1/2	≤0.06-2
ST22 EMRSA-15 (15)	0.12/0.25	≤0.06-0.5	64/>128	025->128	8/32	0.12-32	2/8	≤0.06-8
ST5 Cordobes/Chilian (5)	0.12/-	0.12-1	32/-	16-64	8/-	8-16	2/-	2-4
ST5 USA100 (6)	0.25/-	0.25-1	>128/-	128->128	32/-	32->128	8/-	4-32
USA PFGE types (n)								
USA100 (7)	0.25/-	≤0.06-1	>128/-	16->128	32/-	8->128	8/-	2-32
USA200 (1)	-/-	0.25	-/-	64	-/-	16	-/-	4
USA300 (24)	≤0.06/0.12	≤0.06-0.25	0.25/16	0.25-32	0.5/4	0.12-8	0.12/2	≤0.06-2
USA400 (2)	-/-	≤0.06	-/-	0.25	-/-	0.12-0.25	-/-	≤0.06
USA500 (2)	-/-	0.25	-/-	64	-/-	16	-/-	2-4
USA600 (1)	-/-	0.25	-/-	64	-/-	16	-/-	4
USA700 (1)	-/-	≤0.06	-/-	0.5	-/-	0.25	-/-	≤0.06
USA800 (1)	-/-	≤0.06	-/-	0.5	-/-	0.25	-/-	≤0.06
USA1000 (1)	-/-	≤0.06	-/-	1	-/-	0.25	-/-	≤0.06
USA1100 (1)	-/-	≤0.06	-/-	0.5	-/-	0.25	-/-	≤0.06
Other genetic markers (n)								
pvl positive (8)	≤0.06/-	≤0.06	0.25/-	0.25-1	0.12/-	0.12-0.25	≤0.06/-	≤0.06
*agr*I (4)	-/-	≤0.06-0.25	-/-	0.5-64	-/-	0.25-16	-/-	≤0.06-4
*agr*II (2)	-/-	≤0.06-0.12	-/-	0.5-16	-/-	0.25-8	-/-	≤0.06-2
*agr*III (2)	-/-	≤0.06-0.25	-/-	0.25-64	-/-	0.25-16	-/-	≤0.06-4
*agr*IV (1)	-/-	≤0.06	-/-	1	-/-	0.25	-/-	≤0.06
SCCmec type I (11)	0.12/1	0.12-1	128/>128	16->128	32/>128	8->128	4/32	2-32
SCCmec type II (3)	-/-	0.12-0.25	-/-	16-64	-/-	8-16	-/-	2-4
SCCmec type III (12)	0.25/0.25	0.12-0.25	64/128	8-128	16/32	4-32	4/8	1->128
SCCmec type IV (42)	0.12/0.25	≤0.06-0.5	8/64	0.25->128	4/16	0.12-32	2/8	≤0.06-8
SCCmec type IV-A (3)	-/-	≤0.06	-/-	0.25-2	-/-	0.25-0.5	-/-	≤0.06-0.12

CONCLUSIONS

- Overall, JNJ-Q2 inhibited a panel of 111 major circulating *S. aureus* clones (92.8% MRSA) at MIC values of ≤1 µg/ml ($MIC_{50/90}$, 0.12/0.25 µg/ml). These strains demonstrated high levels of resistance to comparator fluoroquinolones (69.4 to 74.8%) using contemporary CLSI and EUCAST breakpoints.
- JNJ-Q2 inhibited all ten VRSA strains at a MIC of ≤1 µg/ml ($MIC_{50/90}$, 0.25/0.25 µg/ml). All ten strains were resistant to moxifloxacin, levofloxacin and ciprofloxacin.
- JNJ-Q2 retained activity (MIC_{90}, 0.12 to 1 µg/ml) against these sub-sets of challenging strains regardless of antimicrobial resistance phenotype, ST or PFGE clonal types, PVL positivity, and *agr* or SCC*mec* type.

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