

Efficacy of a New Fluoroquinolone, JNJ-Q2, in Murine Models of *Staphylococcus aureus* and *Streptococcus pneumoniae* Skin, Respiratory, and Systemic Infections^{∇§}

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The *in vivo* efficacy of JNJ-Q2, a new broad-spectrum fluoroquinolone (FQ), was evaluated in a murine septicemia model with methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) and in a *Streptococcus pneumoniae* lower respiratory tract infection model. JNJ-Q2 and comparators were also evaluated in an acute murine skin infection model using a community-acquired MRSA strain and in an established skin infection (ESI) model using a hospital-acquired strain, for which the selection of resistant mutants was also determined. JNJ-Q2 demonstrated activity in the MSSA septicemia model that was comparable to that of moxifloxacin (JNJ-Q2 50% effective dose [ED₅₀], 0.2 mg/kg of body weight administered subcutaneously [s.c.] and 2 mg/kg administered orally [p.o.]) and activity in the MRSA septicemia model that was superior to that of vancomycin (JNJ-Q2 ED₅₀, 1.6 mg/kg administered s.c.). In an *S. pneumoniae* lower respiratory tract infection model, JNJ-Q2 displayed activity (ED₅₀, 1.9 mg/kg administered s.c. and 7.4 mg/kg administered p.o.) that was comparable to that of gemifloxacin and superior to that of moxifloxacin. In both MRSA skin infection models, treatment with JNJ-Q2 resulted in dose-dependent reductions in bacterial titers in the skin, with the response to JNJ-Q2 at each dose exceeding the responses of the comparators ciprofloxacin, moxifloxacin, linezolid, and vancomycin. Additionally, in the ESI model, JNJ-Q2 showed a low or nondetectable propensity for ciprofloxacin resistance selection, in contrast to the selection of ciprofloxacin-resistant mutants observed for both ciprofloxacin and moxifloxacin. JNJ-Q2 demonstrated activity that was comparable or superior to the activity of fluoroquinolone or antistaphylococcal comparators in several local and systemic skin infection models performed with both *S. aureus* and *S. pneumoniae* and is currently being evaluated in phase II human clinical trials.

Successive improvements in the spectrum and antimicrobial potency of agents in the fluoroquinolone class have resulted in widespread clinical utility of these agents, and the activities of levofloxacin and moxifloxacin against Gram-positive pathogens, particularly *Streptococcus pneumoniae*, have contributed to the adoption of these agents for the empirical treatment of respiratory tract infections in the community setting. Although fluoroquinolone resistance in *S. pneumoniae* remains low, with the rate of levofloxacin resistance in U.S. isolates typically reported at less than 1% (12), the rate of fluoroquinolone resistance has, in selected populations or geographic regions, been reported to be greater than 10% (1). In association with the introduction of the seven-valent pneumococcal vaccine (PCV7), an increased prevalence in non-PCV7 serotypes has been observed (11, 12), including several predominant fluoroquinolone-resistant and multidrug-resistant clones (4, 10). Sev-

eral of the marketed fluoroquinolone agents also display *in vitro* activity against *Staphylococcus aureus* isolates and have been used successfully to treat staphylococcal infections (31), although none of these marketed agents are approved for the treatment of methicillin-resistant *S. aureus* (MRSA) infections. MRSA has become an increasingly important pathogen in community infections (19), and the increased incidence of infection is associated with elevated resistance, with levofloxacin resistance observed in 70% of recent U.S. clinical MRSA isolates (18). Community staphylococcal isolates typically express elevated levels of several virulence determinants, which are associated with increased virulence in murine models of bacteremia and skin abscess infection (20). Efficacy in murine models of MRSA infection is a key attribute for new antibacterial agents targeted for the treatment of staphylococcal infections, including MRSA infections in the community setting. Several investigational fluoroquinolones active against MRSA (2, 5, 15, 17, 32) are reported to be the subject of ongoing clinical studies that are investigating their efficacy in the treatment of acute bacterial skin and skin structure infections (ABSSI) caused by MRSA. The development of new fluoroquinolone agents retaining activity against multidrug-resistant *S. pneumoniae* isolates and displaying potent antistaphylococcal activities may prove valuable as a therapeutic option for the treatment of respiratory and skin infections. JNJ-Q2 is a new broad-spectrum fluoroquinolone that displays potent *in vitro* activity against *S. pneumoniae*, including

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levofloxacin-resistant and multidrug resistant isolates, and *S. aureus*, including MRSA and ciprofloxacin-resistant MRSA isolates (24; D. J. Farrell, L. C. Liverman, D. J. Biedenbach, and R. N. Jones, JNJ-Q2: a new fluoroquinolone with potent *in vitro* activity against staphylococcus aureus, including methicillin- and fluoroquinolone-resistant strains; poster LB-3, presented at the 48th Annual Meeting of the Infectious Diseases Society of America, 2010) and is currently being evaluated in clinical studies for the treatment of ABSSSI and community-acquired bacterial pneumonia (CABP). JNJ-Q2 displayed lower MIC values than comparator fluoroquinolones against *S. pneumoniae* and *S. aureus* isolates, including MRSA (24), and also displayed a lower propensity for resistance selection with these Gram-positive pathogens (24). Here we report the *in vivo* activities of JNJ-Q2 in murine models of septicemia with MSSA and community-acquired MRSA (CA-MRSA) strains, *S. pneumoniae* lower respiratory tract infections, and MRSA acute and established skin infections. The selection of MRSA mutants with reduced susceptibility to test agents in the established skin infection model is also reported. In an accompanying article, the *in vitro* activities of JNJ-Q2 are presented, highlighting the inhibitory activity against purified target enzymes and *in vitro* biofilms, and *in vitro* resistance development in MRSA (25).

(Portions of this work were previously presented at the 50th Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, 2010 [13].)

MATERIALS AND METHODS

Antimicrobial agents. JNJ-Q2 was synthesized at Johnson & Johnson Pharmaceutical Research & Development, L.L.C. Moxifloxacin was obtained from Bayer AG, gemifloxacin from CB Research and Development, Inc. (New Castle, DE), and linezolid from Organix, Inc. (Wolburn, MA). Ciprofloxacin hydrochloride was obtained from Pentax-Bayer (Kankakee, IL). Vancomycin hydrochloride was purchased from MP Bio (Irvine, CA).

Microorganisms. *S. pneumoniae* (ATCC 6301), *S. aureus* Smith (MSSA; ATCC 13709), and methicillin-resistant *S. aureus* MRSA (ATCC 43300) were purchased from the American Type Culture Collection (ATCC; Manassas, VA). MRSA OC 8525, a community-acquired MRSA strain, was obtained from Barry Kreiswirth of the Public Health Research Institute, Newark, NJ.

Animals. Female CF-1 mice (20 to 22 g) and female Crl:SKH1-hrBr hairless, immunocompetent mice (20 to 25 g) were purchased from Charles River Laboratories (Wilmington, MA), and female Swiss-Webster mice (20 to 22 g) were obtained from Taconic Farms, Inc. (Hudson, NY). Animals were allowed free access to food and water and were maintained on a 12 h light/dark cycle. Mice were allowed to acclimate for 5 days after receipt from the vendor. All animal studies were reviewed and approved by the Johnson & Johnson Pharmaceutical Research & Development Institutional Animal Care and Use Committee. Animal numbers were justified by a power analysis of the treatment group sample size necessary to detect a statistically significant decrease in bacterial CFU or mortality by use of Dunnett's multiple comparison test (33).

Inoculum Preparation. For respiratory studies, *S. pneumoniae* was grown overnight on tryptic soy agar (TSA) containing 5% sheep blood at 35°C in a 5% CO₂ atmosphere. Isolated colonies were inoculated into Trypticase soy broth with 5% heat-inactivated goat serum (Rockland Immunochemicals, Inc., Gilbertsville, PA) and incubated at 35°C until the mid-log growth phase, centrifuged, and then concentrated to approximately 1×10^8 CFU/animal for inoculation. For septicemia studies, overnight cultures of *S. aureus* Smith ATCC 13709 (MSSA) or *S. aureus* OC 8525 (CA-MRSA) were inoculated from frozen glycerol stocks into brain heart infusion (BHI) media and shaken for 18 h at 37°C, centrifuged, and diluted in 7.0% hog gastric mucin (Sigma-Aldrich Chemical Company, St. Louis, MO) in saline solution to approximately 3×10^5 CFU/animal (MSSA) or 1×10^7 CFU/animal (CA-MRSA). In skin studies, overnight cultures of *S. aureus* OC 8525 (CA-MRSA) or MRSA ATCC 43300 were centrifuged as described above and diluted in BHI media with Cytodex microcarrier dextrin beads (Sigma-Aldrich Chemical Company, St. Louis, MO) (131 to 220

μm in diameter) added at a final concentration of 0.1% (vol/vol) to the inoculating dose (7×10^6 CFU/mouse, CA-MRSA, acute skin infection model; 9×10^6 CFU/mouse, MRSA ATCC 43300, established skin infection model).

***In vitro* susceptibility.** MICs were determined by broth microdilution methods according to CLSI guidelines (9).

Mouse septicemia model. To study the comparative efficacies of JNJ-Q2, ciprofloxacin, moxifloxacin, and gemifloxacin in the mouse septicemia model (14, 16), Swiss Webster mice were infected intraperitoneally (i.p.) with *S. aureus* Smith (MSSA) ATCC 13709 (approximately 3×10^5 CFU/mouse, $10\times$ to $100\times$ the 50% lethal dose [LD₅₀]) or with CA-MRSA OC 8525 (approximately 1×10^7 CFU/mouse, $10\times$ to $100\times$ the LD₅₀) and then treated either subcutaneously or orally for 1 h (JNJ-Q2) or 1 and 3 h (comparators) postinfection, and mortality was monitored for 3 days. The dosing regimen (once a day [QD] versus twice a day [BID]) was determined from pilot studies conducted in the mouse septicemia model (data not shown). The 50% effective dose (ED₅₀) and the 90% effective dose (ED₉₀), the doses at which 50% and 90% survival were observed, respectively, and 95% fiducial limits were calculated from the survival curves using a logistical regression. All statistical analyses were carried out in SAS version 5 or 9.1.3 (SAS Institute, Cary, NC).

Lower respiratory tract infection model. To study the comparative efficacies of JNJ-Q2, moxifloxacin, and gemifloxacin in a murine lower respiratory tract infection model (14, 16), female CF-1 mice were briefly anesthetized with isoflurane and infected by placing 50 μl of *S. pneumoniae* ATCC 6301 (approximately 1×10^8 CFU/mouse) at the tip of the nares and allowing the mouse to inhale the inoculum. Mice were then treated subcutaneously or orally once at 24 h postinfection, and mortality was monitored over 2 days. The dose that provided 50% or 90% survival (ED₅₀ or ED₉₀) was calculated from the survival curves using a logistic regression.

Acute skin infection model. To study the comparative efficacies of JNJ-Q2, linezolid, and vancomycin against *S. aureus* in an acute skin infection model (13, 14, 16), female SKH1 mice were anesthetized with isoflurane and given a 0.2-ml subcutaneous (s.c.) injection of a Cytodex bead inoculum containing *S. aureus* (CA-MRSA OC 8525) (approximately 7×10^6 CFU/mouse flank) on the left and right flanks. Animals received JNJ-Q2, linezolid, or vancomycin at 1, 3, 25, and 27 h postinfection at a dose of 1.6, 6.2, 25, or 100 mg/kg of body weight/day. All drugs were delivered orally except for vancomycin, which was given subcutaneously. Animals were euthanized by CO₂ asphyxiation 48 h after infection, and the lesions on each flank were measured. A lesion volume score was calculated from the following equation; $\text{LV} = (\pi/6) (L \times W^2)$, where LV represents the lesion volume, L represents the length of the lesion in millimeters, and W represents the width of the lesion in millimeters (7). For determination of CFU numbers per gram of skin, the skin from the infected areas was disinfected with chlorhexidine diacetate (Nolvasan; Fort Dodge Animal Health, Fort Dodge, IA), excised, weighed, and homogenized in 1 ml of saline solution (Omni Prep tissue homogenizer and Omni Tip disposable rotor stator generator probes; Omni International, Marietta, GA) (4°C and 35,000 rpm for 0.5 min). Serial 100-fold dilutions in saline solution (Remel, Lenexa, KS) (0.85% saline) were plated (Autoplate 4000; Spiral Biotech, Inc., Norwood, MA) on TSA plates. The plates were incubated for 18 h at 37°C and the CFU counted (Q-Count; Spiral Biotech, Inc., Norwood, MA).

Established skin infection model. To study the comparative efficacies of JNJ-Q2, ciprofloxacin, and moxifloxacin against *S. aureus* in an established skin infection model (14), mice were anesthetized with isoflurane and given a 0.2-ml subcutaneous injection of the Cytodex bead inoculum containing *S. aureus* (MRSA ATCC 43300) (approximately 9×10^6 CFU/mouse flank) on the left and right flanks. Then, at 3 days postinfection, twice-daily treatment with JNJ-Q2, ciprofloxacin, or moxifloxacin (25 to 200 mg/kg/day) was initiated, and the treatment was administered for 3 days. All drugs were delivered orally except for ciprofloxacin, which was given intraperitoneally. At 24 h following the last dose (a time point chosen to minimize any drug carryover effect), the animals were euthanized, and skin tissue was processed and analyzed as described previously for the acute skin infection model.

To assess *in vivo* resistance selection, an undiluted skin sample was spiral plated on TSA plates containing ciprofloxacin (2 $\mu\text{g}/\text{ml}$). The plates were incubated for 48 h at 37°C, and the number of CFU was determined. Any colony that grew on a TSA plate containing 2 $\mu\text{g}/\text{ml}$ of ciprofloxacin ($4\times$ the MIC) within 48 h was defined as exhibiting resistance.

Statistical methodology. Preliminary evaluations using descriptive summary statistics suggested mean treatment differences between JNJ-Q2 and comparative treatment groups. Evaluation to determine whether these differences were significant was then performed using logistic regression (for septicemia, lower respiratory tract infection, and resistance selection) (29) or linear mixed-effects modeling (for the acute and established skin infection models) (21) to explain the

TABLE 1. In vitro susceptibility of Gram-positive pathogens to JNJ-Q2 and comparators in the mouse infection models

Drug	MIC ($\mu\text{g/ml}$)			
	<i>S. pneumoniae</i> ATCC 6301	MSSA ATCC 13709	MRSA OC 8525	MRSA ATCC 43300
JNJ-Q2	0.015	0.008	0.015	0.004
Ciprofloxacin	0.25	0.25	2	0.5
Moxifloxacin	0.25	0.06	0.25	0.03
Gemifloxacin	0.03	0.008	0.06	0.03
Linezolid	1	4	4	2
Vancomycin	0.5	1	1	1

response ratios as a function of log-transformed doses and treatment groups. The modeling was adjusted to account for overdispersion as appropriate.

Further comparisons of the effects of JNJ-Q2 seen with comparative treatments were assessed by using a linear-contrast argument within this model. Additionally, modeling diagnostics were provided for assessing goodness of fit. Differences were considered significant at the 0.05 level.

RESULTS

In vitro susceptibility. The MICs for JNJ-Q2 and all comparators against *S. pneumoniae* ATCC 6301, *S. aureus* Smith (MSSA; ATCC 13709), and methicillin-resistant *S. aureus* (MRSA; OC 8525 and ATCC 43300) are shown in Table 1. Against *S. pneumoniae*, JNJ-Q2 was 2-, 16-, 16-, 32-, and 64-fold more potent than gemifloxacin, ciprofloxacin, moxifloxacin, vancomycin, and linezolid, respectively. Against MSSA, JNJ-Q2 and gemifloxacin were equipotent, and both were 8-, 32-, 128-, and 512-fold more potent than moxifloxacin, ciprofloxacin, vancomycin, and linezolid, respectively. Against MRSA, JNJ-Q2 was 4- to 128-fold more potent than the fluoroquinolone comparators and 64- to 512-fold more potent than the anti-MRSA comparators linezolid and vancomycin.

Mouse septicemia model. The activities (ED_{50} s and ED_{90} s) of JNJ-Q2, moxifloxacin, and gemifloxacin are summarized in Table 2. In the systemic infection model used with *S. aureus* Smith, JNJ-Q2 was 6- and 9-fold more potent than ciprofloxacin by the oral ($P < 0.0021$) and subcutaneous ($P < 0.0001$) routes of administration, respectively. JNJ-Q2 displayed ED_{50} values that were similar to moxifloxacin and gemifloxacin values by the subcutaneous route of administration. However, JNJ-Q2 was more potent ($P < 0.0001$) than gemifloxacin according to comparisons of the slopes of the dose-response profiles. When the compounds were administered orally, ED_{50} values for JNJ-Q2 were similar to moxifloxacin values ($P > 0.85$), but JNJ-Q2 was slightly less potent than gemifloxacin ($P > 0.14$).

JNJ-Q2 was also compared to the anti-MRSA comparators linezolid and vancomycin with respect to activity against a CA-MRSA (OC 8525) strain in the murine septicemia model (Table 2). The activity (ED_{50}) of JNJ-Q2 administered orally was less than that of linezolid ($P < 0.0087$) in this model; however, JNJ-Q2 was 2- and 8-fold more active than linezolid ($P < 0.0004$) and vancomycin ($P < 0.0001$), respectively, when the compounds were administered subcutaneously.

Lower respiratory tract infection model. The efficacies (ED_{50} s and ED_{90} s) of JNJ-Q2, moxifloxacin, and gemifloxacin in the mouse *S. pneumoniae* lower respiratory tract infection model are detailed in Table 3. JNJ-Q2 was 2- and 10-fold more

TABLE 2. In vivo efficacy of single doses of JNJ-Q2 and comparators in a murine septicemia model with *S. aureus* Smith (MSSA) or OC 8525 (CA-MRSA)

Compound, organism category, and dosing route (n^a)	ED_{50} in mg/kg/day ^b (95% fiducial limits)	ED_{90} in mg/kg/day ^c (95% fiducial limits)	P value ^d
JNJ-Q2			
MSSA			
p.o. (24)	2.0 (1.5–2.5)	4.0 (3.2–7.9)	NA
s.c. (16)	0.15 (0.05–0.22)	0.4 (0.3–0.8)	NA
CA-MRSA			
p.o. (16)	12 (9.1–20.4)	33.7 (21.1–108.6)	NA
s.c. (16)	1.6 (1.0–2.2)	5.4 (3.4–10.6)	NA
Ciprofloxacin			
MSSA			
p.o. (16)	11 (6.5–41)	31.0 (NL)	<0.0021
s.c. (16)	1.4 (0.92–2.5)	3.3 (NL)	<0.0001
Moxifloxacin			
MSSA			
p.o. (13)	1.5 (0.73–2.2)	3.1 (2.2–7.6)	>0.85
s.c. (13)	0.4 (0.2–0.8)	2.8 (1.5–9.7)	<0.0001
Gemifloxacin			
MSSA			
p.o. (21)	1.1 (0.82–1.4)	2.8 (1.4–3,036)	>0.14
s.c. (13)	0.1 (0.06–0.14)	0.2 (0.15–0.45)	<0.0001
Linezolid ^e			
CA-MRSA			
p.o. (18)	5.1 (3–8)	16.6 (11.9–31.5)	<0.0087
s.c. (18)	3.7 (2–6)	11.6 (8.3–22.8)	<0.0004
Vancomycin ^e			
CA-MRSA			
s.c. (32)	12 (10–14)	40.8 (29.1–69.4)	<0.0001

^a n , number of animals per group.

^b ED_{50} s calculated using SAS version 5.

^c ED_{90} s calculated using SAS version 9.1. NL, no limits obtained.

^d P values represent measurements of the significance of comparisons of dose-response profiles of survival incidence data for JNJ-Q2 and comparators. NA, not applicable.

^e Dosed BID, 1 and 3 h postinfection.

active than moxifloxacin by the oral ($P < 0.0086$) and subcutaneous ($P < 0.0001$) routes of administration, respectively. JNJ-Q2 displayed activity similar to that of gemifloxacin when administered either subcutaneously or orally in this model.

Acute skin infection model. The effects of JNJ-Q2, linezolid, and vancomycin on acute skin infections mediated by a CA-MRSA strain (OC 8525) are shown in Fig. 1. In untreated control animals, the starting inoculum of approximately $6.8 \log_{10}$ CFU increased to $7.5 \log_{10}$ CFU ($8 \log_{10}$ CFU/g skin tissue) during the 48-h testing period. At every dose tested (1.6 to 100 mg/kg/day), JNJ-Q2 displayed greater reductions in bacterial burden in the skin of mice than linezolid ($P < 0.0001$) or vancomycin ($P < 0.0045$). At the highest dose tested (100 mg/kg/day), JNJ-Q2 reduced the bacterial burden in the skin by 2.5 and 1.3 \log_{10} CFU/g more than linezolid and vancomy-

TABLE 3. In vivo efficacy of single doses of JNJ-Q2 and comparators in a murine lower respiratory tract infection model with *S. pneumoniae* ATCC 6301

Compound and dosing route (n ^a)	ED ₅₀ in mg/kg/day ^b (95% fiducial limits)	ED ₉₀ in mg/kg/day ^c (95% fiducial limits)	P value ^d
JNJ-Q2			
p.o. (16)	7.4 (5.3–10.1)	19.7 (13.8–38.0)	NA
s.c. (16)	1.9 (0.7–3.3)	18.3 (9.5–80.3)	NA
Moxifloxacin			
p.o. (16)	14 (8–23)	41.5 (28.3–84.2)	<0.0086
s.c. (16)	23 (NL)	NATC	<0.0001
Gemifloxacin			
p.o. (16)	3.9 (2.2–5.8)	25.7 (16.4–53.2)	<0.0066
s.c. (16)	2.0 (1.2–2.9)	15.2 (8.9–40.6)	<0.0104

^a n, number of animals per group.
^b ED₅₀s calculated using SAS version 5. NL, no limits obtained.
^c ED₉₀s calculated using SAS version 9.1. NATC, program not able to calculate.
^d P values represent measurements of the significance of comparisons of dose-response profiles of survival incidence data for JNJ-Q2 and comparators. NA, not applicable.

cin, respectively, and reduced the bacterial titer by nearly 3 log₁₀ CFU/g from the titer seen with the starting inoculum.

The skin lesion volumes resulting from infection with the CA-MRSA strain are shown in Fig. 2. The reductions in lesion volume were concordant with the reductions in CFU (Fig. 1). Animals treated with JNJ-Q2 had the smallest lesion volumes at every dose compared to those resulting from treatment with linezolid (P < 0.0001) and vancomycin (P < 0.0001).

Established skin infection model. The efficacies of JNJ-Q2, ciprofloxacin, and moxifloxacin in mice with established (3-day) skin infections due to MRSA ATCC 43300 are shown in Fig. 3. Bacterial burdens in untreated control mice increased 1.4 log₁₀ CFU to 7.7 log₁₀ CFU (8.2 log₁₀ CFU/g skin tissue) over the 6-day testing period. Oral treatment with JNJ-Q2 at 25, 50, 100, and 200 mg/kg/day resulted in dose-dependent reductions of 0.7, 1.1, 2.4, and 3.0 log₁₀ CFU/g skin tissue. In contrast, treatment with either ciprofloxacin (P < 0.0007) or moxifloxacin (P < 0.0379) did not result in reductions in CFU below the initial infecting inocula (6.8 log₁₀ CFU). At 200

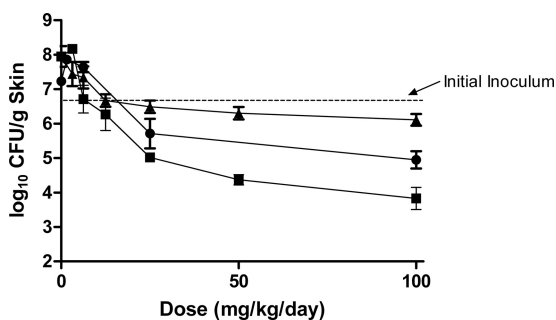


FIG. 1. The effect of JNJ-Q2 (p.o., squares), linezolid (p.o., triangles), and vancomycin (s.c., circles) on acute CA-MRSA (OC 8525) skin infections. Each symbol represents the mean ± standard error for 8 to 20 mice. The starting inoculum was 6.8 log₁₀ CFU/mouse, and untreated control values (48 h) were 8.0 log₁₀ CFU/g skin tissue. The P values were generated by comparing the slopes of the dose-response curves for JNJ-Q2 versus linezolid (P value, <0.0001) and for JNJ-Q2 versus vancomycin (P value, <0.0045).

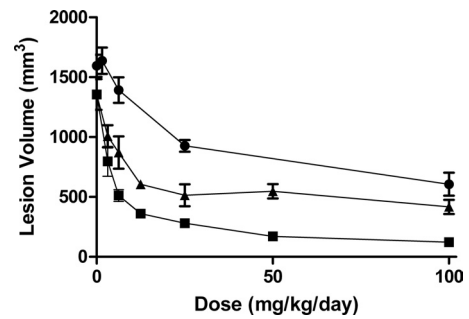


FIG. 2. The effect of JNJ-Q2 (p.o., squares), linezolid (p.o., triangles), and vancomycin (s.c., circles) on lesion volume in mice infected with CA-MRSA (OC 8525) in the acute skin infection model. Each symbol represents the mean ± standard error for 8 to 20 mice. The starting inoculum was 6.8 log₁₀ CFU/mouse, and untreated control values (48 h) were 8.0 log₁₀ CFU/g skin tissue. The P values were generated by comparing the slopes of the dose-response curves for JNJ-Q2 versus linezolid (P value, <0.0001) and for JNJ-Q2 versus vancomycin (P value, <0.0001) (note that the modeling contains a term for quadratic dose determinations).

mg/kg/day, the highest dose tested, JNJ-Q2 treatment resulted in 2.8- and 1.9-fold greater reductions of MRSA numbers in the skin of mice than ciprofloxacin and moxifloxacin, respectively.

Skin lesion volumes for mice infected with MRSA ATCC 43300 and treated with JNJ-Q2, ciprofloxacin, or moxifloxacin are shown in Fig. 4. Control mice had a mean lesion volume of 611 mm³. Treatment with JNJ-Q2 resulted in lesion volumes that were 46 to 63% smaller than those in control mice, in comparison with reductions in lesion volume of 19 to 46% and 19 to 28% for ciprofloxacin (P < 0.0092) and moxifloxacin (P < 0.0001), respectively.

The propensities for JNJ-Q2, ciprofloxacin, and moxifloxacin to select for ciprofloxacin resistance in the established mouse skin infection model are summarized in Table 4. No resistant colonies were detected in any of the samples after

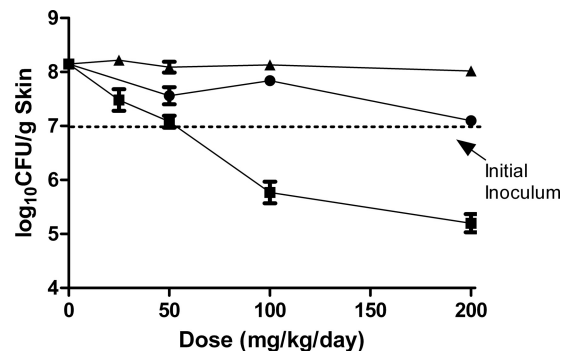


FIG. 3. The effect of JNJ-Q2 (p.o., squares), ciprofloxacin (i.p., triangles), and moxifloxacin (p.o., circles) on established MRSA ATCC 43300 skin infections. Each symbol represents the mean ± standard error for 14 to 16 mice. The starting inoculum was 7.0 log₁₀ CFU/mouse, and untreated control values (3 days) were 8.2 log₁₀ CFU/g skin tissue. The P values were generated by comparing the slopes of the dose-response curves for JNJ-Q2 versus ciprofloxacin (P value, <0.0007) and for JNJ-Q2 versus moxifloxacin (P value, <0.0379) (note that the modeling contains a term for dose * treatment interaction determinations).

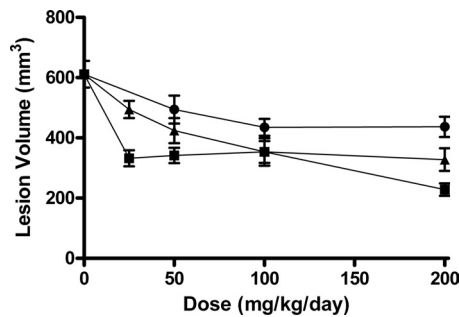


FIG. 4. The effect of JNJ-Q2 (p.o., squares), ciprofloxacin (i.p., triangles), and moxifloxacin (p.o., circles) on lesion volume in mice with established MRSA ATCC 43300 skin infections. Each symbol represents the mean \pm standard error for 14 to 16 mice. The starting inoculum was $7.0 \log_{10}$ CFU/mouse, and untreated control values (3 days) were $8.2 \log_{10}$ CFU/g skin tissue. The *P* values were generated by comparing the slopes of the dose-response curves for JNJ-Q2 versus ciprofloxacin (*P* value, <0.0092) and for JNJ-Q2 versus moxifloxacin (*P* value, <0.0001) (note that the modeling contains a term for quadratic dose determinations).

treatment with JNJ-Q2 at 50 to 200 mg/kg/day. Samples from animals treated with JNJ-Q2 at 25 mg/kg/day contained low levels of resistant colonies, which grew in the presence of 2 μ g/ml ciprofloxacin in 3/16 skin samples; however, counts were below the limit of reliable detection. In contrast, resistant colonies were recovered from each dose group of animals receiving ciprofloxacin. The density of resistant bacterial cells averaged approximately $3 \log_{10}$ CFU/g skin tissue in each ciprofloxacin dose group. Ciprofloxacin-resistant colonies were also cultured from samples taken from moxifloxacin-treated animals (50 to 200 mg/kg/day), with resistant bacterial densities ranging from 2.6 to $3.2 \log_{10}$ CFU/g skin tissue. The selection of resistance in infected animals treated with ciprofloxacin or moxifloxacin was statistically significant (*P* < 0.001) compared to the incidence of resistance following treatment of animals with JNJ-Q2.

DISCUSSION

The activity of the new fluoroquinolone JNJ-Q2 compared to the activity of other fluoroquinolones and anti-MRSA comparators in murine models of systemic, respiratory, and localized skin infections was assessed. The activities assessed included the efficacy of JNJ-Q2 in treating murine systemic and skin infections caused by MRSA and an established skin infection model with a virulent CA-MRSA isolate.

The fluoroquinolone moxifloxacin has demonstrated increased utility in treating respiratory tract infections, as it is associated with high rates of microbiological success in the clinic (3). *In vitro*, moxifloxacin displays activity against *S. pneumoniae*, including some ciprofloxacin-resistant isolates carrying quinolone resistance-determining region (QRDR) mutations; however, the MICs of JNJ-Q2 were 32-fold lower than those of moxifloxacin for these *S. pneumoniae* isolates (24). The greater *in vitro* potency of JNJ-Q2 was reflected in the relative activities of these agents in the murine lower respiratory tract infection model, in which JNJ-Q2 displayed lower ED₅₀ and ED₉₀ values than moxifloxacin.

The increased *in vitro* activity of JNJ-Q2, in comparison with

other fluoroquinolone agents, against *S. aureus*, including MRSA and ciprofloxacin-resistant MRSA (24), was likewise reflected in the murine septicemia model with *S. aureus* MSSA and MRSA strains. Against MSSA, peroral ED₅₀ values for JNJ-Q2 were comparable to moxifloxacin values, although JNJ-Q2 was more active by the subcutaneous route of administration. The moxifloxacin ED₅₀ values for *S. aureus* Smith in our study closely matched those published previously for a systemic infection model (27). In the septicemia model with MRSA, JNJ-Q2 was more active than either of the anti-MRSA agents linezolid or vancomycin.

JNJ-Q2 exhibited dose-dependent reductions in bacterial load in an established MRSA mouse skin infection model, with limited evidence of resistance selection. In the MRSA established skin infection model, no resistant colonies were selected by JNJ-Q2 at doses of 50 to 200 mg/kg/day. This was in contrast to the comparator fluoroquinolone agents ciprofloxacin and moxifloxacin, which both selected for ciprofloxacin-resistant colonies within infected skin at the same doses. Poor efficacy in the established skin infection model observed in ciprofloxacin-treated animals was possibly due to the selection of resistant bacteria during treatment. Resistance selection in ciprofloxacin-treated animals was extensive, with 70 to 100% of treated mice yielding resistant colonies from infected skin samples. Given the clinical experience of ciprofloxacin, against which resistance in MRSA was observed to emerge rapidly (6), the reduced potential for resistance selection is a key attribute for a new fluoroquinolone developed to treat MRSA infections. Weight loss was noted in the high-ciprofloxacin-dose group (data not shown), which precluded increasing the dose to further evaluate efficacy. Concordant with our efficacy results, Cagni and colleagues (8) reported only minimal activity with ciprofloxacin (administered BID for 7 days) in a 21-day established rat tissue cage infection model; in contrast to our study, however, they did not isolate any resistant colonies following therapy. The difference in resistance selection observed here in the skin infection model reflects differences in the *in vitro* resistance rates observed for JNJ-Q2 and ciprofloxacin with MRSA, including isolates carrying QRDR mutations and displaying elevated ciprofloxacin MICs (24). Minimal efficacy was noted with moxifloxacin, even when administered at 200 mg/kg/day. As seen with ciprofloxacin treatment, resistant isolates were recovered at every dose level tested, but to a lesser extent, which may have negatively impacted the efficacy of moxifloxa-

TABLE 4. Incidence of ciprofloxacin-resistant bacteria arising within ATCC 43300 (MRSA) mouse skin abscesses following treatment with JNJ-Q2 or comparators

Dose (mg/kg/day)	No. of resistant colonies/total no. of colonies ^a		
	JNJ-Q2 ^b	Ciprofloxacin	Moxifloxacin
25	3/16	16/16	NT ^c
50	0/16	16/16	5/16
100	0/14	11/16	7/16
200	0/14	11/16	2/16

^a Values represent the total number of samples that had any MRSA growth on a TSA plate containing ciprofloxacin (2 μ g/ml) versus the total number of samples tested.

^b *P* < 0.001 versus ciprofloxacin and moxifloxacin by logistic regression analysis.

^c NT, not tested.

cin in this setting. This model of an established skin infection was fairly robust in that the infection involved a bacterial population of $8 \log_{10}$ CFU/g tissue ($7.7 \log_{10}$ CFU total), permitting the differentiation of agents with disparate propensities for resistance selection.

In both the acute and established skin infection models, the observed reductions in lesion volume were generally dose dependent; however, lesions that were smaller overall were observed in animals in the established skin infection model. This may be reflective of staphylococcal infections being self-limiting in these murine models and could in part be due to the reported enhanced ability of *S. aureus* to bind hemoglobin derived from humans compared to that derived from other mammals, including mice (28).

The increased efficacy of JNJ-Q2 in comparison to ciprofloxacin and moxifloxacin in several mouse models of infection may have resulted from the lower MIC values of JNJ-Q2 for the infecting strains, and the pharmacokinetic exposures of JNJ-Q2 in the mouse underscore the potency of this compound. JNJ-Q2 is 67% bound to mouse plasma proteins and was 8% orally bioavailable in the mouse (S. Steller and A. Streeter, unpublished data). This compares to mouse oral bioavailability of 38% and 78% for ciprofloxacin and moxifloxacin, respectively (22, 30). JNJ-Q2 was 65% orally bioavailable in dogs, monkeys (G. Eichenbaum and S. Stellar, unpublished data), and humans (J. M. Davenport, P. Covington, L. Liverman, G. McIntyre, and J. Almenoff, presented at the Annual Meeting of the American College of Clinical Pharmacology, Chicago, IL, 2011). In the mouse, an oral dose of JNJ-Q2 administered at 10 mg/kg yielded an area under the curve (AUC) of $0.13 \mu\text{g} \cdot \text{h/ml}$ (S. Steller and A. Streeter, unpublished data), a value that is 11-fold and 6-fold lower than the values seen with oral ciprofloxacin and moxifloxacin mouse exposures, respectively (23, 27). In the established skin infection model, studies with ciprofloxacin and moxifloxacin included doses of 150 and 200 mg/kg, respectively, achieving exposures in the mouse comparable to human doses of 750 and 400 mg, respectively (23, 26, 27). In the septicemia model, the lower ED₅₀ value for the subcutaneous administration of JNJ-Q2 in comparison to that for moxifloxacin reflects the lower MIC values for JNJ-Q2, but the lower ED₅₀ values for moxifloxacin following peroral administration likely resulted from its greater oral bioavailability in the mouse. In contrast, in the lower respiratory tract infection model, JNJ-Q2 displayed lower ED₅₀ values than moxifloxacin by both oral and subcutaneous routes of administration, possibly resulting from increased *in vitro* potency and the potential for increased lung exposure for JNJ-Q2.

In conclusion, JNJ-Q2 displayed promising levels of efficacy in a variety of local and systemic mouse infection models and warrants further study.

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