

# In Vitro Activity of WCK 771, a Benzoquinolizine Fluoroquinolone (Levonadifloxacin) when Tested Against Contemporary Gram-Positive and -Negative Bacteria from a Global Surveillance Program

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### Introduction

Levonadifloxacin is the S-(-) isomer of the benzoquinolizine fluoroquinolone nadifloxacin and is two- to four-fold more active than the racemic mixture. Intravenous (WCK 771) and oral (WCK 2349) formulations of this anti-methicillin-resistant *Staphylococcus aureus* (MRSA) fluoroquinolone, levonadifloxacin are in clinical development and were awarded QIDP status. US Phase 1 studies for both the dosage forms have been completed and recently WCK 2349 underwent QT, hepatic impairment and intrapulmonary concentrations determination studies.

Levonadifloxacin has a broad-spectrum of *in vitro* activity including fluoroquinolone-resistant MRSA. In this study, levonadifloxacin was tested against Gram-positive and -negative clinical isolates collected in medical centers worldwide as part of the 2014 SENTRY Antimicrobial Surveillance Program.

### Methods

**Susceptibility testing:** MIC values were determined using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology as described in CLSI document M07-A10 (2015). Frozen-form MIC panels (96-well; produced by JMI Laboratories [North Liberty, Iowa, USA]) were used to test levonadifloxacin and levofloxacin. Cation-adjusted Mueller-Hinton broth (CA-MHB) was used for inoculating MIC panels for all non-fastidious organisms. *Haemophilus influenzae* was tested in Haemophilus Test Medium (HTM). In addition, lysed horse blood (2.5-5%) was utilized to supplement the CA-MHB for testing of *Streptococcus pneumoniae*, viridans group streptococci and β-hemolytic streptococci. MIC values for comparator agents were obtained from the SENTRY Antimicrobial Surveillance Program database.

Comparator agents were tested by CLSI broth microdilution methods using validated dry-form panels prepared by ThermoFisher Scientific (Cleveland, Ohio, USA). Quality control (QC) strains were tested daily and inoculum density was monitored by colony counts. QC ranges and interpretive criteria for the comparator compounds was as published in CLSI M100-S26 (2016); tested QC strains included the following: *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

**Isolates:** A total of 12,474 bacterial isolates collected from 154 medical institutions located in four continents (United States [69 centers], Europe [44], Latin America [20], Asia-Pacific [21]) were selected from the global surveillance program. The collection included 7,569 Gram-positive and 4,905 Gram-negative organisms. All isolates were collected in 2014, except for *Streptococcus* spp., *H. influenzae* and *M. catarrhalis* from Latin America, which included isolates collected in 2013 and 2014. Only clinically significant isolates were included in the study (one per patient episode). Species identification was confirmed when necessary by Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS) using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, USA) following manufacturer instructions. Isolates were obtained from patients hospitalized with pneumonia (17.8%; 2,220), community-acquired pneumonia (18.1%; 2,255), bloodstream infections (19.6%; 2,447), urinary tract infections (5.5%; 684), acute bacterial skin and soft tissue infections, e.g. wound swabs or aspirated pus etc. (28.9%; 3,610), intra-abdominal infections (2.4%; 302) and other infection types (7.7%; 956).

### Results

#### Activity against Gram-positive bacteria

- Levonadifloxacin demonstrated good activity overall ( $\text{MIC}_{50/90}$ : 0.015/1 µg/mL) against 4,077 *S. aureus* with 96.8% of all isolates (91.5% of MRSA) inhibited at ≤2 µg/mL, which is a suggested breakpoint based on PK/PD (Bhagwat et al., 2009) (Table 1). Isolates with levonadifloxacin MIC values >2 µg/mL occurred in all regions among methicillin-resistant (MR) isolates. These MR isolates with levonadifloxacin MICs >2 µg/mL were also levofloxacin- and ciprofloxacin-resistant ( $\text{MIC} > 4$  µg/mL).
- A total of 88.8% of levofloxacin-nonsusceptible strains of *S. aureus* were inhibited at a levonadifloxacin MIC of ≤2 µg/mL (Table 1). Similar to other anti-staphylococcal quinolones, the potency of levonadifloxacin was lower in levofloxacin-nonsusceptible strains of *S. aureus* compared to levofloxacin-susceptible strains (levonadifloxacin  $\text{MIC}_{90}$ : 4 µg/mL and 0.015 µg/mL, respectively (Table 1)). Similar results were noted in all four geographic regions.
- Similar to coagulase positive staphylococci, levonadifloxacin demonstrated potent activity ( $\text{MIC}_{50/90}$ : 0.06/1 µg/mL) against 620 coagulase negative staphylococci with 93.4% of all isolates inhibited at a PK-PD breakpoint of ≤2 µg/mL of (Table 1). A total of 90.7% of MRCoNs and 98.6% of MSCoNs were inhibited at a levonadifloxacin MIC of ≤2 µg/mL (Table 1). Similar to other quinolones, the potency of levonadifloxacin was observed to be lower in oxacillin- (methicillin-) resistant coagulase negative staphylococci compared to methicillin-susceptible (MS); levonadifloxacin  $\text{MIC}_{90}$ : 2 µg/mL and 0.06 µg/mL, respectively) see Table 1.
- Levonadifloxacin was very active ( $\text{MIC}_{50/90}$ : 0.25/0.5 µg/mL) against all 1,196 *S. pneumoniae* isolates with 98.6% of isolates inhibited at MIC values of ≤2 µg/mL (Table 1). Identical  $\text{MIC}_{50/90}$  values were observed in each of the four geographic regions. Levonadifloxacin  $\text{MIC}_{50/90}$  values (0.25/0.5 µg/mL) were two- to four-fold lower than those of levofloxacin (1/1 µg/mL; Table 2).

- Comparative agents were tested by CLSI broth microdilution methods using validated dry-form panels prepared by ThermoFisher Scientific (Cleveland, Ohio, USA). Quality control (QC) strains were tested daily and inoculum density was monitored by colony counts. QC ranges and interpretive criteria for the comparator compounds was as published in CLSI M100-S26 (2016); tested QC strains included the following: *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.
- Isolates:** A total of 12,474 bacterial isolates collected from 154 medical institutions located in four continents (United States [69 centers], Europe [44], Latin America [20], Asia-Pacific [21]) were selected from the global surveillance program. The collection included 7,569 Gram-positive and 4,905 Gram-negative organisms. All isolates were collected in 2014, except for *Streptococcus* spp., *H. influenzae* and *M. catarrhalis* from Latin America, which included isolates collected in 2013 and 2014. Only clinically significant isolates were included in the study (one per patient episode). Species identification was confirmed when necessary by Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS) using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, USA) following manufacturer instructions. Isolates were obtained from patients hospitalized with pneumonia (17.8%; 2,220), community-acquired pneumonia (18.1%; 2,255), bloodstream infections (19.6%; 2,447), urinary tract infections (5.5%; 684), acute bacterial skin and soft tissue infections, e.g. wound swabs or aspirated pus etc. (28.9%; 3,610), intra-abdominal infections (2.4%; 302) and other infection types (7.7%; 956).

### Conclusions

- Levonadifloxacin demonstrated excellent activity against all 504 *M. catarrhalis* isolates ( $\text{MIC}_{50/90}$ : 0.015/0.015 µg/mL; Table 3) with inhibition of all isolates observed at an MIC of ≤0.25 µg/mL.
- Levonadifloxacin demonstrated similar activities against all *E. coli* ( $\text{MIC}_{50/90}$ : 0.25/≤8 µg/mL; Table 3), *Klebsiella* spp. ( $\text{MIC}_{50/90}$ : 0.5/≤8 µg/mL), *K. pneumoniae* ( $\text{MIC}_{50/90}$ : 0.5/≤8 µg/mL), *K. oxytoca* ( $\text{MIC}_{50/90}$ : 0.5/4 µg/mL), *Proteus mirabilis* ( $\text{MIC}_{50/90}$ : 0.5/≤8 µg/mL), *Citrobacter* spp. ( $\text{MIC}_{50/90}$ : 0.25/8 µg/mL), *Enterobacter* spp. ( $\text{MIC}_{50/90}$ : 0.25/8 µg/mL), and indole-positive *Proteus* spp. ( $\text{MIC}_{50/90}$ : 0.5/≤8 µg/mL). For 664 isolates of *K. pneumoniae*, a total of 66.3% of isolates were inhibited at a levonadifloxacin MIC value of ≤4 µg/mL (Table 1). Ciprofloxacin resistance was at 27.7-33.0% (CLSI/EUCAST; data not shown). Ciprofloxacin resistance ranged from 11.6% (CLSI) in the USA to 56.6% (EUCAST) in Latin America (data not shown).
- Against the Gram-positive organisms, levonadifloxacin was highly active against *S. aureus* with 96.8% of all isolates and 91.5% of MRSA inhibited at a MIC of ≤2 µg/mL. Against CoNS, 93.4% of isolates were inhibited at levonadifloxacin MIC values of ≤2 µg/mL and for *S. pneumoniae*, 98.6% of isolates were inhibited at levonadifloxacin MIC values of ≤2 µg/mL.
- Against the Gram-negative respiratory pathogens *H. influenzae* and *M. catarrhalis*, levonadifloxacin was highly active with MIC<sub>90</sub> values at 0.015-0.03 µg/mL and activity was similar against β-lactamase-positive and -negative isolates.
- Against Enterobacteriaceae, 73.2% of isolates were inhibited at levonadifloxacin concentrations of ≤4 µg/mL ( $\text{MIC}_{50/90}$ : 0.5/≤8 µg/mL) and levonadifloxacin was less active against ESBL-phenotypes than non-ESBL-phenotypes. A total of 67.4% of *P. aeruginosa* isolates were inhibited at levonadifloxacin concentrations of ≤4 µg/mL ( $\text{MIC}_{50/90}$ : 2/≤8 µg/mL).
- Levonadifloxacin's broad-spectrum activity, including potent activity against MRSA, supports the potential value of further development studies to define its clinical role.

Table 2. Activity of levonadifloxacin and comparator antimicrobial agents when tested against selected Gram-positive isolates.

Organism (no. total)	MIC (µg/mL) $\text{MIC}_{50}$	$\text{MIC}_{90}$	Range	%S	%I	%R	%S	%I	%R
<b><i>S. aureus</i> (4,077)</b>									
Levonadifloxacin	0.015	1	≤0.004 → 8	-	-	-	-	-	-
Levofloxacin	0.25	>8	0.03 → 8	71.7	0.1	28.1	71.7	0.1	28.1
Ciprofloxacin	0.5	>4	≤0.03 → 4	69.5	2.1	28.4	69.5	-	30.5
Clinamycin	≤0.25	>2	≤0.25 → 2	86.7	0.1	13.2	86.3	0.4	13.3
Daptomycin	0.25	0.5	≤0.06 → 4	99.9	-	-	99.9	-	0.1
Erythromycin	0.25	16	≤0.12 → 16	58.7	4.7	36.9	58.7	1.2	39.6
Gentamicin	≤1	≤1	≤1 → 8	93.1	0.4	6.6	92.8	-	7.2
Cefazidime	0.25	16	≤0.12 → 16	57.7	9.1	2.2	57.7	4.2	19.0
Ceftriaxone	0.12	8	≤0.06 → 8	75.0	1.5	23.5	75.0	1.5	23.5
Ciprofloxacin	≤0.03	4	≤0.03 → 4	75.0	2.2	22.9	73.0	2.0	25.0
Gentamicin	≤1	8	≤1 → 8	85.7	1.1	13.3	84.5	1.2	14.3
Tetracycline	≤0.5	1	≤0.5 → 8	91.9	0.8	7.3	90.8	0.5	8.7
Tigecycline	0.06	0.06	≤0.015 → 0.5	100.0	-	-	100.0	-	0.0
Ciprofloxacin	≤0.5	5	≤0.5 → 4	98.7	1.3	9.8	97.0	0.3	1.0
Meropenem	≤0.03	0.06	≤0.015 → 0.32	97.3	0.3	2.4	97.6	1.0	1.4
Piperacillin-tazobactam	2	32	≤0.5 → 64	88.1	5.2	6.7	84.6	3.5	11.9

Table 3. Activity of levonadifloxacin and comparator antimicrobial agents when tested against selected Gram-negative isolates.

Organism (no. tested)	MIC (µg/mL) $\text{MIC}_{50}$	$\text{MIC}_{90}$	Range	%S	%I	%R	%S	%I	%R
<b><i>Enterobacteriaceae</i> (3,000)</b>									
Levonadifloxacin	0.5	>8	0.015 → 8	-	-	-	-	-	-
Levofloxacin	0.06	>8	≤0.008 → 8	78.1	1.2	20.1	76.9	1.2	21.9
Amikacin	2	4	≤0.25 → 32	98.1	1.1	0.8	97.0	1.1	1.9
Aztreonam	≤0.12	16	≤0.12 → 16	79.8	2.1	18.1	77.0	2.8	20.2
Cefepime	≤0.5	16	≤0.5 → 16	83.9	3.4	12.8*	81.9	3.5	14.6
Ceftazidime	0.25	16	≤0.12 → 16	81.0	1.6	16.7	76.7	4.2	19.0
Ceftriaxone	0.12	8	≤0.06 → 8	75.0	1.5	23.5	75.0	1.5	23.5
Ciprofloxacin	≤0.03	4	≤0.03 → 4	75.0	2.2	22.9	73.0	2.0	25.0
Gentamicin	≤1	8	≤1 → 8	85.7	1.1	13.3	84.5	1.2	14.3
Imipenem	≤0.12	1	≤0.12 → 8	91.4	5.4	3.2	96.8	1.9	1.3
Meropenem	≤0.03	0.06	≤0.015 → 1	97.3	0.3	2.4	97.6	1.0	1.4
Piperacillin-tazobactam	2	32	≤0.5 → 64	88.1	5.2	6.7	84.6	3.5	11.9
<b><i>E. coli</i> (1,058)</b>				</					