

# In Vitro activity of a Novel Benzoquinolizine Antibiotic, Levonadifloxacin (WCK 771) against Blood Stream Gram-Positive Isolates from a Tertiary Care Hospital

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## Abstract

**Background** Blood stream infections (BSIs) due to Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) are associated with high mortality ranging from 10 to 60%. The current anti-MRSA agents have limitations with regards to safety and tolerability profile which limits their prolonged usage. Levonadifloxacin and its oral prodrug alalevonadifloxacin, a novel benzoquinolizine antibiotic, have recently been approved for acute bacterial skin and skin structure infections including diabetic foot infections and concurrent bacteremia in India.

**Methods** The present study assessed the potency of levonadifloxacin, a novel benzoquinolizine antibiotic, against Gram-positive blood stream clinical isolates ( $n = 31$ ) collected from January to June 2019 at a tertiary care hospital in Mumbai, India. The susceptibility of isolates to antibacterial agents was defined following the Clinical and Laboratory Standard Institute interpretive criteria (M100 E29).

**Results** High prevalence of MRSA (62.5%), quinolone-resistant *Staphylococcus aureus* (QRSA) (87.5%), and methicillin-resistant coagulase-negative staphylococci (MR-CoNS) (82.35%) were observed among bacteremic isolates. Levonadifloxacin demonstrated potent activity against MRSA, QRSA, and MR-CoNS strains with significantly lower minimum inhibitory concentration MIC<sub>50/90</sub> values of 0.5/1 mg/L as compared with levofloxacin (8/32 mg/L) and moxifloxacin (2/8 mg/L).

**Conclusion** Potent bactericidal activity coupled with low MICs support usage of levonadifloxacin for the management of BSIs caused by multidrug resistant Gram-positive bacteria.

## Keywords

- ▶ blood stream infections
- ▶ methicillin-resistant *S. aureus*
- ▶ levonadifloxacin
- ▶ quinolone-resistant *S. aureus*

## Introduction

With more than 90 years from the discovery of penicillin, bacterial infections are still the leading cause of morbidity

and mortality worldwide including India. Blood stream infections (BSIs) caused by Gram-positive pathogens may originate from diverse sources such as pneumonia, urinary tract infection, acute bacterial skin and skin structure

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infection (ABSSSI), and catheter-associated infections. In the event of suboptimal treatment, BSI may result in further complications such as infective endocarditis and sepsis. Mortality rates ranging from 40 to 60% and prolonged length of intensive care unit stay have been observed with BSI.<sup>1</sup> The Indian Network for Surveillance of Antimicrobial Resistance has reported the methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia rates to be 48%.<sup>2</sup> Further, infection with MRSA is found to be an independent predictor of mortality in BSI.<sup>3,4</sup>

Management of MRSA in India, especially in BSI is quite challenging. This can be attributed to ineffectiveness of the current anti-MRSA agents (e.g., vancomycin, linezolid, daptomycin). Both vancomycin and linezolid lack rapid cidal action which restricts their clinical utility in the treatment of BSI. Despite the availability of vancomycin, which is a standard therapy for BSI management, the mortality rates were reported to be > 30%.<sup>5</sup> Reports have listed vancomycin minimum inhibitory concentration (MIC) creep, variable tissue penetration, and nephrotoxicity at high serum concentrations, as major challenges in its clinical use.<sup>6</sup> Linezolid is also not indicated to be used in staphylococcal bacteremia due to its bacteriostatic mode of action. Moreover, prolonged usage of linezolid is not approved because of adverse effects such as thrombocytopenia, optic, and peripheral neuropathy.<sup>7</sup> On the other hand, daptomycin, even though cidal, is ineffective in pneumonia (a major source for gram-positive BSI) as it is inactivated by pulmonary surfactant<sup>8</sup> and is also shown to have cross-resistance with vancomycin in heteroresistant vancomycin-intermediate *Staphylococcus aureus* (hVISA) strains.<sup>9</sup> Hence, there is a need for a new drug having rapid bactericidal action, low probability of resistance development, and lower risk of side effects, to achieve maximum microbiological and clinical cure in BSI.

Levonadifloxacin-IV (WCK 771) and its oral L-alanine prodrug, alalevonadifloxacin (WCK 2349), are novel broad spectrum antibiotics belonging to benzoquinolizine subclass of fluoroquinolone, approved in India for the indication of ABSSSI with concurrent bacteremia and diabetic foot infections caused by susceptible Gram-positive pathogens. The distinctive novel structural feature of a nonbasic hydroxypiperidine side chain at C-8 position of tricyclic benzoquinolizine core confers a well differentiated mode of action to levonadifloxacin as compared with other quinolones. It primarily targets deoxyribonucleic acid gyrase which allows it to demonstrate high potency even against commonly prevalent quinolone-resistant *Staphylococcus aureus* (QRSA) that harbors multiple mutations in topoisomerase

IV.<sup>10</sup> Levonadifloxacin not only exhibit bactericidal effect on biofilm-embedded QRSA and MRSA but also inhibits NorA efflux pump which is a major cause of quinolone resistance development. Moreover, levonadifloxacin has an effective cidal action against high density cultures.<sup>11</sup> In the present study, we tested in vitro activity of levonadifloxacin against BSI gram-positive clinical isolates.

## Methods

The study was conducted between January and June 2019 at S.L. Raheja Hospital, Mumbai, Maharashtra, India, and has been approved by the institutional ethics committee (CLS/2017/SLRH). The study isolates (*n* = 31) were collected as nonduplicate samples from individual patients suspected with BSI potentially caused by Gram-positive pathogens. Organisms responsible for BSI were later identified by biochemical and molecular methods. The identified organisms included *S. epidermidis* (*n* = 17), *S. aureus* (*n* = 8), and *Enterococci* spp. (*n* = 6).

## Susceptibility Testing

The MICs of levonadifloxacin and other comparator antibiotics were determined by the reference agar dilution method as described by the Clinical and Laboratory Standard Institute (CLSI).<sup>12</sup> Susceptibility of levonadifloxacin was defined on the basis of susceptibility breakpoint of 2 mg/L for Gram-positive pathogens. The susceptibility of other antibacterial agents was defined following interpretive criteria presented in the CLSI document M100 edition 29.<sup>13</sup> *S. aureus* ATCC 29213 was included in each run as quality control strain.

## Results and Discussion

Out of 31 isolates, 54.8% were caused by *S. epidermidis* followed by *S. aureus* (25.8%) and *Enterococci* (19.3%). Among these clinical isolates, high prevalence of MRSA (62.5%), QRSA (87.5%), and methicillin-resistant coagulase-negative staphylococci (MR-CoNS, 82.35%) was observed. Levonadifloxacin showed best-in-class activity against collected *Staphylococci* isolates (*n* = 25) which included MRSA (*n* = 8), QRSA (*n* = 8), and MR-CoNS (*n* = 17). Levonadifloxacin demonstrated significantly lower MIC<sub>50/90</sub> values of 0.5/1 mg/L as compared with levofloxacin (8/32 mg/L) and moxifloxacin (2/8 mg/L) against these isolates (► **Table 1**). ► **Table 2** illustrates MIC values of levonadifloxacin in comparison to vancomycin, linezolid, and teicoplanin against *Staphylococci* isolates.

**Table 1** Cumulative distribution of MICs of levonadifloxacin in comparison to levofloxacin and moxifloxacin

Antibacterial	% cumulative inhibition of <i>Staphylococci</i> spp. at respective MIC (mg/L)											
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32
Levonadifloxacin (WCK 771)	3 (12.0)	3 (24.0)	0 (24.0)	0 (24.0)	8 (56.0)	9 (92.0)	2 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Levofloxacin	0	0	1 (4.0)	3 (16.0)	1 (20.0)	1 (24.0)	0 (24.0)	5 (44.0)	9 (80.0)	2 (88.0)	2 (96.0)	1 (100)
Moxifloxacin	0	3 (12.0)	0 (12.0)	3 (24.0)	0 (24.0)	4 (40.0)	9 (76.0)	3 (88.0)	3 (100)	0 (100)	0 (100)	0 (100)

Abbreviation: MIC, minimum inhibitory concentration.

**Table 2** Susceptibility profiling of levonadifloxacin and comparators against Staphylococci spp.

Antibacterial agent	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	S	I	R
Levonadifloxacin (WCK 771)	0.03–2	0.5	1	100 <sup>a</sup>	–	–
Moxifloxacin	0.06–8	2	2	24	16	60
Levofloxacin	0.12–> 32	8	8	24	0	76
Vancomycin	0.5–2	1	1	100	0	0
Linezolid	0.5–8	1	1	96	–	4
Teicoplanin	0.5–16	2	2	96	4	0
Co-trimoxazole	0.06–> 32	0.5	0.5	80	–	20

Abbreviation: MIC, minimum inhibitory concentration.

<sup>a</sup>Susceptibility of levonadifloxacin was defined on the bases of susceptibility break point of 2 mg/L.

Enterococci are known to display high level of resistance to most of the antibiotic classes. In such challenging scenarios, levonadifloxacin MICs were found to be lower (2–16 mg/L) than moxifloxacin (2–32 mg/L) and levofloxacin (> 32 mg/L). The potency of comparator anti-MRSA drugs vancomycin (MIC range 0.5–16 mg/L) and linezolid (MIC range 1–8 mg/L) were in expected range.

In the past, several susceptibility testing studies undertaken with levonadifloxacin demonstrated consistent MIC<sub>90</sub> of 0.5 to 1 mg/L against variety of Gram-positive pathogens. For instance, its potent activity has been reported against 1,438 MRSA isolates with MIC<sub>90</sub> of 1 mg/L in a global surveillance study.<sup>14</sup> Similarly, against 308 QRSA isolates collected in a recent multicenter study, conducted in India, MIC<sub>90</sub> of 1 mg/L was reported in comparison to the MIC<sub>90</sub> of 16 mg/L for levofloxacin.<sup>15</sup> Therefore, the present study result are in agreement with the previous studies which substantiate the consistent activity profile of levonadifloxacin. In summary, potent bactericidal activity of levonadifloxacin coupled with promising activity observed in the present study support clinical use of levonadifloxacin for difficult-to-treat Gram-positive BSIs.

#### Authors' Contribution

R.R., P.J., and P.B.: Data generation and analysis, drafting of the manuscript, and critical revision. D.M., S.S., J.A., and R.J.: Study concept and design, study supervision, data interpretation, critical revision, and final approval of the manuscript.

#### Conflict of Interest

D.M., S.S., and P.B. have nothing to declare. R.R., P.J., J.A., and R.J. are employees of Wockhardt Ltd.

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