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Activity of the new quinolone WCK 771 against pneumococci

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ABSTRACT

The activity of WCK 771, a new experimental quinolone being developed to overcome quinolone resistance in staphylococci, against quinolone-susceptible and -resistant pneumococci was determined. Comparative activities of ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, clinafloxacin, vancomycin, linezolid, amoxycillin, cefuroxime, azithromycin and clarithromycin were determined with MIC and time-kill experiments. Animal experiments were also performed to test the in-vivo anti-pneumococcal activity of WCK 771 compared to levofloxacin. WCK 771 $MIC_{50/90}$ values for 300 quinolone-susceptible *Streptococcus pneumoniae* isolates (108 penicillin-susceptible, 92 penicillin-intermediate and 100 penicillin-resistant) were 0.5/0.5 mg/L; the MICs of β-lactams and macrolides rose with those of penicillin G, and all isolates were susceptible to vancomycin and linezolid. WCK 771 $MIC_{50/90}$ values for 25 quinolone-resistant pneumococcal isolates were 4/8 mg/L, compared to 0.5/1 mg/L for clinafloxacin, 2/4 mg/L for gatifloxacin and moxifloxacin, 8/16 mg/L for levofloxacin, and 16/>32 mg/L for ciprofloxacin. Time-kill studies showed that WCK 771 was bactericidal against pneumococci after 24 h at 4× MIC, as were the other quinolones tested. Animal model studies showed that WCK 771 had efficacy comparable to that of levofloxacin, by both the oral and subcutaneous routes, for systemic infection caused by three quinolone-susceptible isolates of pneumococci. Overall, WCK 771 was potent both *in vivo* and *in vitro* against quinolone-susceptible, but not quinolone-resistant, *S. pneumoniae*, regardless of penicillin susceptibility.

Keywords Activity, new antibiotics, quinolones, *Streptococcus pneumoniae*, WCK 771

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INTRODUCTION

As the prevalence of multiresistant strains of *Streptococcus pneumoniae* has increased worldwide, there has been an attendant need for new antimicrobial agents. Introduced in the 1980s, fluoroquinolones fulfilled this need initially, and these agents are still important for the treatment of a wide range of infections. However, resistance to many members of this class of agent is emerging in pneumococci [1,2], although the prevalence of resistance remains low (< 2%) in most parts of the world [2–7]. Pneumococcal resistance to penicillin G and other β-lactam and non-β-lactam compounds has also increased worldwide, including in the USA. Major foci of resistance include South Africa, Spain and central and eastern Europe [3–5]. In the USA, surveys have shown an increase in resistance to penicillin (including resistance classed as penicillin-intermediate) from < 5% before 1989 to 6.6% in 1991–1992 and, more recently, to 28.7–37% [6,7]. The problem of drug-resistant pneumococci is compounded by the spread of resistant clones from country to country and worldwide [8–10].

There is a need for oral compounds for outpatient treatment of respiratory tract infections caused by penicillin- and macrolide-resistant pneumococci [11,12]. Older quinolones, such as ciprofloxacin and ofloxacin, have only moderate activity *in vitro* against pneumococci, with MICs

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clustering around resistance breakpoints. Newer quinolones, such as levofloxacin, gatifloxacin, moxifloxacin and gemifloxacin, have greater anti-pneumococcal activity than the older agents [4,5,13–19]. However, recent reports from Hong Kong [20], Canada [21] and Spain [22] have described an increasing prevalence of quinolone-resistant pneumococci. Quinolone resistance in *S. pneumoniae* is mediated by stepwise changes in the quinolone resistance-determining regions of type II topoisomerase; mutations in *parC* and *gyrA* are commonest, but *parE* and *gyrB* mutations are also encountered [2]. The prevalence of resistant strains is likely to increase with increased use of broad-spectrum quinolones for empirical therapy of community-acquired respiratory tract infections.

WCK 771 (Fig. 1; Wockhardt Research Centre, Aurangabad, India), the hydrate of the arginine salt of *S*-(–)-nadifloxacin, is a new experimental quinolone with excellent anti-staphylococcal activity that is undergoing phase I studies in India as a parenteral antibacterial agent. The present study sought to shed more light on the activity of WCK 771 against Gram-positive bacteria by examining its activity against *S. pneumoniae* isolates with differing susceptibilities to penicillin G and quinolones in comparison with ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, clinafloxacin, vancomycin and linezolid. Additionally, nine pneumococcal isolates were tested in time-kill experiments with all six quinolones. Finally, the in-vivo efficacy of WCK 771 was assessed in comparison with levofloxacin in a mouse systemic infection model with three quinolone-susceptible pneumococcal isolates, as well as in a lung pneumococcal load-reduction study.

MATERIALS AND METHODS

Bacterial isolates

The isolates tested comprised 300 quinolone-susceptible and 25 quinolone-resistant *S. pneumonia*e. All isolates were from clinical specimens (sputum, bronchial and tracheal aspirates, eye cultures, blood, cerebrospinal fluid) obtained from patients from throughout the USA and countries in western Europe during 1998–2002. Quinolone-susceptible isolates were defined as those with ciprofloxacin MICs ≤2.0 mg/L, and quinolone-resistant isolates as those with MICs ≥4 mg/L [23]. Among the 300 quinolone-susceptible isolates, 108 were penicillin-susceptible (MICs ≤0.06 mg/L), 92 were penicillin-intermediate (MICs 0.12–1.0 mg/L), and 100 were penicillin-resistant (MICs 2.0–16.0 mg/L). All penicillin-susceptible pneumococci were recent isolates from the USA, while penicillin-intermediate and -resistant pneumococci were recent isolates from the USA, South Africa, Spain, France, central and eastern Europe and Korea. The 25 quinolone-resistant pneumococcal isolates were selected from our collection. Mechanisms of quinolone resistance for these isolates included alterations in the quinolone-resistance-determining regions of ParC, GyrA, ParE and/or GyrB. Mutations in *par*C were at S79F, S79Y, D83N, D83G, N91D, R95C or K137N. Mutations in *gyrA* were at S81A, S81C, S81F, S81Y, E85K or S114G. Nineteen isolates had a mutation in *parE* at D435N or I460V. Only one isolate had a mutation in *gyrB* at E474K. Nineteen isolates had a total of three or four mutations in the quinolone-resistance-determining regions of *parC*, *gyrA*, *parE*, and/or *gyrB*.

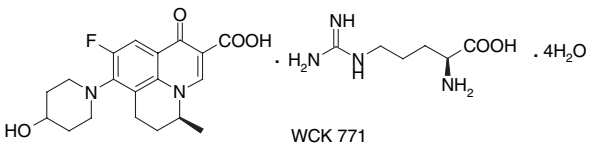

Fig. 1. Chemical structure of WCK 771.

Antimicrobial agents and MIC testing

WCK 771 was synthesised at Wockhardt Research Centre, Aurangabad, India. Other antimicrobial agents were either synthesised at Wockhardt Research Centre (clinafloxacin) or obtained from their respective manufacturers. Agar dilution testing was performed on Mueller–Hinton agar (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with sheep blood 5% v/v, with incubation in air for 24 h [23]. MICs of the pneumococci tested with time-kill kinetics were determined by broth microdilution in Mueller–Hinton broth (BBL Microbiology Systems) supplemented with sheep blood 5% v/v. Standard quality control strains, including *S. pneumoniae* ATCC 49619, were included in each batch of agar or broth microdilution tests [23]. Data were interpreted according to standard recommendations [24].

Determination of the efflux mechanism of quinolone-resistant pneumococci

Quinolone MICs for quinolone-resistant pneumococci were determined in the presence and absence of reserpine (Sigma, St Louis, MO, USA) 10 mg/L as described previously [25–27]. The agents tested were WCK 771, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin and clinafloxacin. The existence of an efflux system was recognised by a quinolone MIC that was at least four-fold lower in the presence of reserpine compared to the MIC without reserpine. Testing was repeated three times.

Time-kill testing

The time-kill activity of quinolones was tested against nine selected pneumococcal isolates, in Mueller–Hinton broth with lysed horse blood 5% v/v, as described previously [28]. The isolates tested included three penicillin-susceptible, three penicillin-intermediate and three penicillin-resistant isolates. One of the penicillin-resistant isolates was also quinolone-resistant, with a ciprofloxacin MIC of 32 mg/L. This latter isolate had mutations in *gyrA* (S81Y), *parC* (S79F, K137N) and *parE* (I460V).

Antibiotic concentrations were chosen to provide three doubling dilutions above and one dilution below the previously determined MIC. Growth controls with inoculum but no antibiotic were included in each experiment [28]. The original viable count was determined with use of the untreated growth control. Only inocula within the range 5×10^5 - 5×10^6 CFU/mL were acceptable [28], and testing of out-of-range samples was repeated. Viability counts of antibiotic-containing suspensions were performed at 0, 3, 6 and 24 h. Colony counts were performed on plates yielding 30–300 colonies. The lower sensitivity limit of colony counts was 300 CFU/mL [28].

The results of time-kill assays were analysed by determining the number of strains which yielded $\Delta\log_{10}$ CFU/mL reductions of −1, −2 and −3 at 3, 6, 12 and 24 h respectively, compared to baseline counts (0 h). Antimicrobial agents were considered bactericidal at the lowest concentration that reduced the original inoculum by ≥3 $\log_{10}$ CFU/mL (99.9%) at each of the time periods, and bacteriostatic if the inoculum was reduced by <3 $\log_{10}$ CFU/mL. The problem of drug carryover was addressed by dilution, as described previously [28].

Systemic infection model

The comparative in-vivo efficacies of WCK 771 and levofloxacin were studied in an intraperitoneal mouse septicaemia model with the use of three quinolone-susceptible *S. pneumoniae* strains, SPN 727, SPN 731 and SPN 733. Treatment was given 1 h and 4 h post-infection by the subcutaneous and oral routes for each respective group. Survival was monitored until day 7, and 50% effective dose (ED_{50}) and 90% effective dose (ED_{90}) values with 95% confidence intervals were calculated by probit analysis [29] and the method of Litchfield and Wilcoxin [30], respectively.

Lung load-reduction study

Ten Swiss mice weighing 18–22 g were infected (3×10^4 CFU/animal) with *S. pneumoniae* strain 6303 (type 3) by the intraperitoneal route. Treatment was given 1 h and 4 h post-infection with an oral dose of either 75 or 100 mg/kg, twice-daily for 2 days, for both WCK 771 and levofloxacin. The animals were killed humanely 24 h after the last dose, and lungs were excised and homogenised in 5 mL of chilled saline. Viable counts in lung homogenates were determined in terms of lung load/animal. The percentage of animals showing sterile lungs (a count of ≤10 CFU/mL) was calculated for WCK 771 and levofloxacin (Fig. 2).

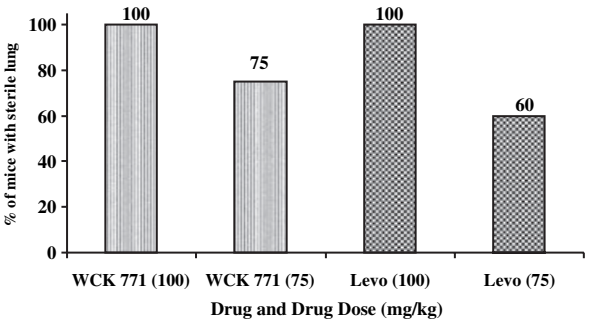

Fig. 2. Percentage of mice with sterile lungs following exposure to *Streptococcus pneumoniae* strain 6303. MIC (mg/L): WCK 771, 0.25; levofloxacin (Levo), 1.0. Route of infection: intraperitoneal. Route of treatment: oral.

Table 1. Agar dilution MICs (mg/L) for 300 quinolone-susceptible *Streptococcus pneumoniae* isolates[a]

Antimicrobial agent	MIC range	MIC_{50}	MIC_{90}
WCK 771	0.12–1	0.5	0.5
Ciprofloxacin	0.25–2	1	2
Levofloxacin	0.5–2	1	1
Gatifloxacin	0.06–0.25	0.25	0.25
Moxifloxacin	0.06–0.5	0.12	0.25
Clinafloxacin	0.016–0.25	0.12	0.12
Penicillin[b]			
Penicillin S	≤ 0.008–0.06	0.03	0.03
Penicillin I	0.12–1	0.25	1.0
Penicillin R	2–16	2	4
Amoxycillin			
Penicillin S	≤ 0.008–0.06	0.016	0.03
Penicillin I	0.03–1	0.12	1
Penicillin R	0.5–16	2	4
Cefuroxime			
Penicillin S	≤ 0.008–2	0.03	0.06
Penicillin I	0.12–4	0.5	2
Penicillin R	2–32	4	16
Azithromycin			
Penicillin S	≤ 0.008 to > 64	0.06	4
Penicillin I	0.03 to > 64	0.06	> 64
Penicillin R	0.03 to > 64	> 64	> 64
Clarithromycin			
Penicillin S	≤ 0.008to > 64	0.03	2
Penicillin I	0.016 to > 64	0.03	64
Penicillin R	0.016 to > 64	8	> 64
Vancomycin	0.06–0.5	0.25	0.5
Linezolid	0.03–2	1	2

[a]Ciprofloxacin MICs ≤ 2.0 mg/L.
[b]108 penicillin-susceptible, 92 penicillin-intermediate and 100 penicillin-resistant isolates.

RESULTS

MICs for the 300 quinolone-susceptible *S. pneumoniae* isolates are presented in Table 1. Clinafloxacin had the lowest MICs of all quinolones tested (MIC_{50} and MIC_{90} both 0.12 mg/L), followed by moxifloxacin (0.12/0.25 mg/L), gatifloxacin (0.25/0.25 mg/L), WCK 771 (0.5/0.5 mg/L), levofloxacin (1/1 mg/L) and ciprofloxacin (1/2 mg/L). MICs of β-lactams and macrolides rose with those of penicillin G, and all isolates were susceptible to vancomycin and linezolid.

For the 25 quinolone-resistant pneumococcal isolates (ciprofloxacin MICs ≥4 mg/L), clinafloxacin had the lowest MICs (range 0.25–1.0 mg/L, $MIC_{50/90}$ 0.5/1 mg/L). MICs of the other quinolones ranged between 0.25 and >32 mg/L, with $MIC_{50/90}$ values of 2/4 mg/L for moxifloxacin, 2/4 mg/L for gatifloxacin, 4/8 mg/L for WCK 771, 8/16 mg/L for levofloxacin, and 16/> 32 mg/L for ciprofloxacin (Table 2). In 12 of the 25 quinolone-resistant isolates, evidence was found for the presence of an efflux mechanism for

Table 2. Agar dilution MICs (mg/L) for 25 ciprofloxacin-resistant *Streptococcus pneumoniae* isolates[a]

Quinolone	MIC range	MIC_{50}	MIC_{90}
WCK 771	0.25–8.0	4.0	8.0
Ciprofloxacin	4.0 to > 32.0	16.0	> 32.0
Levofloxacin	4.0–32.0	8.0	16.0
Gatifloxacin	0.25–4.0	2.0	4.0
Moxifloxacin	0.25–4.0	2.0	4.0
Clinafloxacin	0.25–1.0	0.5	1.0

[a]Ciprofloxacin MICs ≥ 4.0 mg/L.

Table 3. MICs for *Streptococcus pneumoniae* isolates tested in time-kill experiments (n = 9), including one quinolone-resistant isolate

	MICs (mg/L) for each isolate								
Agent	1	2	3	4	5	6	7	8	9
Penicillin G	0.03	≤ 0.015	≤ 0.015	0.12	0.25	0.5	2.0	4.0	4.0
WCK 771	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	8.0
Ciprofloxacin	1.0	1.0	1.0	1.0	2.0	2.0	1.0	1.0	32.0
Levofloxacin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	16.0
Gatifloxacin	0.25	0.25	0.25	0.25	0.5	0.5	0.25	0.25	8.0
Moxifloxacin	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	4.0
Clinafloxacin	0.06	0.06	0.06	0.06	0.12	0.12	0.06	0.06	0.5

some of the quinolones tested, but MICs of WCK 771 were unaffected by the presence of reserpine. In the presence of reserpine, 11 of the 25 isolates had lower ciprofloxacin MICs (four- to 16-fold), three had lower clinafloxacin MICs (four- to eight-fold), two had lower gatifloxacin and levofloxacin MICs (four-fold), and one had a lower moxifloxacin MIC (four-fold).

Microdilution MICs for the nine *S. pneumoniae* isolates tested in time-kill experiments are presented in Table 3. Time-kill analysis showed that WCK 771 was bactericidal (99.9% killing) at 2× MIC after 24 h with all nine pneumococcal isolates tested, including the quinolone-resistant isolate

Table 4. *Streptococcus pneumoniae* time-kill results for nine isolates, showing the numbers of isolates with 1, 2 and 3 $\log_{10}$ decreases in viable counts in relation to MICs at the time-points indicated

	3 h			6 h			12 h			24 h		
Agent	− 1[a]	− 2[a]	− 3[a]	− 1	− 2	− 3	− 1	− 2	− 3	− 1	− 2	− 3
WCK 771A												
4× MIC	9	7	0	9	9	2	9	9	7	9	9	9
2× MIC	9	4	0	9	8	2	9	9	6	9	9	9
MIC	7	3	0	9	5	0	9	8	6	8	5	3
Ciprofloxacin												
4× MIC	9	5	0	9	9	2	9	9	6	9	9	9
2× MIC	9	4	0	9	6	1	9	9	5	9	9	8
MIC	6	0	0	8	5	0	9	8	4	6	3	2
Levofloxacin												
4× MIC	9	6	0	9	9	2	9	9	6	9	9	9
2× MIC	8	4	0	9	6	2	9	8	5	9	9	9
MIC	7	3	0	9	6	0	9	7	4	9	8	6
Gatifloxacin												
4× MIC	9	5	0	9	9	2	9	9	6	9	9	9
2× MIC	9	3	0	9	6	0	9	9	5	9	9	9
MIC	5	0	0	8	2	0	8	8	4	8	8	3
Moxifloxacin												
4× MIC	9	5	0	9	7	2	9	9	7	9	9	9
2× MIC	9	1	0	9	4	1	9	9	7	9	9	9
MIC	3	0	0	5	0	0	9	7	2	6	6	2
Clinafloxacin												
4× MIC	9	6	1	9	9	4	9	9	7	9	9	9
2× MIC	9	3	0	9	8	2	9	9	6	9	9	9
MIC	4	0	0	8	2	0	9	8	3	7	6	2

[a]90%, 99%, 99.9% killing.

with defined mutations in type II topoisomerase (Table 4). Other quinolones gave similar time-kill kinetics relative to their differing MICs.

The comparative in-vivo efficacies of WCK 771 and levofloxacin against three pneumococcal isolates are shown in Table 5. The efficacy of WCK 771 administered by the subcutaneous and oral routes was comparable to that of levofloxacin, with both having ED_{50} values in the range 3–50 mg/kg. In a lung load-reduction study with *S. pneumoniae* strain 6303 and an oral dose of 75 mg/kg (Fig. 2), levofloxacin resulted in sterile

Table 5. In-vivo efficacy of WCK 771 for the treatment of *Streptococcus pneumoniae* infections[a]

			Effective subcutaneous dose (mg/kg) (95% CI)		Effective oral dose (mg/kg) (95% CI)	
Strain	Quinolone	MIC (mg/L)[b]	ED_{50}	ED_{90}	ED_{50}	ED_{90}
SPN 727 (type 19)	WCK 771	0.125	3.6 (1.2–8.1)	9.3 (4.5–21.3)	11.8 (8.5–16.5)	28.65 (20.3–40.3)
	Levofloxacin	1.0	3.2 (1.4–3.9)	9.5 (5.1–10.8)	5.4 (1.3–21.7)	23.19 (13.3–40.3)
SPN 731 (type 14)	WCK 771	0.25	10.0 (3.2–25.5)	20.0 (13.4–64.5)	17.8 (7.0–45.4)	53.12 (36.6–76.97)
	Levofloxacin	1.0	7.5 (1.0–15.8)	15.0 (5.0–46.9)	15.9 (12.5–20.1)	25.6 (19.3–34.13)
SPN 733 (type 7B)	WCK 771	0.25	50 (28.4–53.12)	75 (39.5–188.6)	45.5 (37.0–56.1)	84.4 (58.2–122.2)
	Levofloxacin	1.0	50 (22.4–57.8)	75 (40.1–147.9)	43.3 (36.4–51.5)	71.3 (56–90.7)

[a]Route of infection: intraperitoneal. Infecting dose: 2–3 × 10^8 CFU/animal. Treatment: 1 h and 4 h post-infection. Observation period: 7 days. Endpoint: percentage survival on day 7.
[b]MIC determination by agar dilution.

lungs in 60% of animals, compared with 40% for WCK 771; however, at a dose of 100 mg/kg, both WCK 771 and levofloxacin resulted in 100% of animals having sterile lungs.

DISCUSSION

WCK 771 is an experimental quinolone that is being developed for clinical use. Preliminary data presented in 2001 indicated that WCK 771 has improved potency against staphylococci, including methicillin-resistant strains, compared to other quinolones (41st Interscience Conference on Antimicrobial Agents and Chemotherapy, abstracts F-539, F-541 and F-542). MIC_{50} and MIC_{90} values (mg/L) of WCK 771 for quinolone-susceptible staphylococci were 0.008–0.015 and 0.015–0.03, compared to levofloxacin values of 0.125 and 0.25, respectively. Against quinolone-resistant staphylococci, WCK 771 MIC_{50} and MIC_{90} values (mg/L) were 0.5 and 1, compared to 8 and 32 for levofloxacin, respectively (abstract F-542), while anti-pneumococcal MICs were reported to be about one dilution lower than those of levofloxacin (abstract F-541). The results of the current study supported these preliminary findings, in that anti-pneumococcal MICs were one or two dilutions lower than those of levofloxacin. MICs of other quinolones for *S. pneumoniae* were consistent with those reported previously [4,5,15–19,31,32]. MICs of non-quinolone agents against pneumococci were similar to those described previously, with higher cefuroxime and macrolide MICs for isolates with raised penicillin MICs [4,5,16–19].

In the present study, clinafloxacin, which is no longer being developed, had the lowest MICs of the agents tested for all pneumococcal isolates, followed by moxifloxacin, gatifloxacin, WCK 771, levofloxacin and ciprofloxacin. Quinolone efflux was present in 12 of the 25 quinolone-resistant pneumococcal isolates studied, mainly affecting ciprofloxacin, which is consistent with published data regarding this mechanism in *c.* 50% of quinolone-resistant pneumococcal isolates [33]. The activity of WCK 771 was not affected by the presence of a quinolone efflux mechanism in quinolone-resistant *S. pneumoniae* isolates. However, the activity of WCK 771 against quinolone-resistant pneumococci ($MIC_{50/90}$ values of 4/8 mg/L) was not as good as its activity against quinolone-susceptible pneumococci ($MIC_{50/90}$ values of 0.5/0.5 mg/L). Thus, the activity of WCK 771 against quinolone-resistant pneumococci is four- to eight-fold lower than that against quinolone-resistant staphylococci ($MIC_{50/90}$ values of 0.5/1 mg/L).

The results of time-kill studies showed that WCK 771 was bactericidal if pneumococci, including the one quinolone-resistant isolate tested, were exposed to this agent for 24 h. The bactericidal activities of other quinolones were similar to those found in previous studies [19,28,34,35].

The results of the animal model studies suggested that the activity of WCK 771 against pneumococci was comparable to that of levofloxacin, with ED_{50} values of 3–50 mg/kg for both agents by both the oral and subcutaneous routes in a systemic mouse infection model, and with comparable results being obtained with 100 mg/kg for both agents in pneumococcal lung load-reduction studies.

In summary, WCK 771 showed superior potency to older agents (ciprofloxacin, levofloxacin) and similar potency to newer agents (gatifloxacin, moxifloxacin) against quinolone-susceptible pneumococci. However, as with other quinolones, activity against quinolone-resistant isolates was considerably lower. During the dose-escalation stage of phase I studies, a favourable safety and human pharmacokinetic profile resulted in sustained drug levels above the MIC_{90} for quinolone-susceptible pneumococci, as well as methicillin-resistant staphylococci. On the basis of these observations, WCK 771 has the potential to provide coverage against methicillin-resistant staphylococci as well as quinolone-susceptible pneumococci.

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