Antipneumococcal Activity of DW-224a, a New Quinolone, Compared to Those of Eight Other Agents

Klaudia Kosowska-Shick,¹ Kim Credito,¹ Glenn A. Pankuch,¹ Gengrong Lin,¹
Bülent Bozdogan,¹ Pamela McGhee,¹ Bonifacio Dewasse,¹ Dong-Rack Choi,²
Jei Man Ryu,² and Peter C. Appelbaum^{1*}

*Department of Medicine, Hershey Medical Center, Hershey, Pennsylvania 17033,¹
and Dong Wha Pharmaceuticals, Inc., Anyang City, Republic of Korea²*

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DW-224a is a new broad-spectrum quinolone with excellent antipneumococcal activity. Agar dilution MIC was used to test the activity of DW-224a compared to those of penicillin, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, gemifloxacin, amoxicillin-clavulanate, cefuroxime, and azithromycin against 353 quinolone-susceptible pneumococci. The MICs of 29 quinolone-resistant pneumococci with defined quinolone resistance mechanisms against seven quinolones and an efflux mechanism were also tested. DW-224a was the most potent quinolone against quinolone-susceptible pneumococci (MIC₅₀, 0.016 µg/ml; MIC₉₀, 0.03 µg/ml), followed by gemifloxacin, moxifloxacin, gatifloxacin, levofloxacin, and ciprofloxacin. β-Lactam MICs rose with those of penicillin G, and azithromycin resistance was seen mainly in strains with raised penicillin G MICs. Against the 29 quinolone-resistant strains, DW-224a had the lowest MICs (0.06 to 1 µg/ml) compared to those of gemifloxacin, clinafloxacin, moxifloxacin, gatifloxacin, levofloxacin, and ciprofloxacin. DW-224a at 2× MIC was bactericidal after 24 h against eight of nine strains tested. Other quinolones gave similar kill kinetics relative to higher MICs. Serial passages of nine strains in the presence of sub-MIC concentrations of DW-224a, moxifloxacin, levofloxacin, ciprofloxacin, gatifloxacin, gemifloxacin, amoxicillin-clavulanate, cefuroxime, and azithromycin were performed. DW-224a yielded resistant clones similar to moxifloxacin and gemifloxacin but also yielded lower MICs. Azithromycin selected resistant clones in three of the five parents tested. Amoxicillin-clavulanate and cefuroxime did not yield resistant clones after 50 days.

The incidence of pneumococci resistant to penicillin G and other β-lactam and non-β-lactam compounds has increased worldwide, including in the United States, at an alarming rate. Major foci of infections currently include South Africa, Spain, and central and Eastern Europe (1, 15–17). In the United States, a recent survey has shown an increase in resistance to penicillin from <5% before 1989 (including <0.02% of isolates with MICs of ≥2.0 µg/ml) to 6.6% from 1991 to 1992 (with 1.3% of isolates with MICs of ≥2.0 µg/ml) (3). In another more recent survey, 50.4% of 1,476 clinically significant pneumococcal isolates were not susceptible to penicillin (17). It is also important to note the high rates of isolation of penicillin-intermediate and -resistant pneumococci (approximately 30%) in middle ear fluids from patients with refractory otitis media compared to those from other isolation sites (2). The problem of drug-resistant pneumococci is compounded by the ability of resistant clones to spread from country to country and continent to continent (24, 25).

There is an urgent need of oral compounds for outpatient treatment of otitis media and respiratory tract infections caused by penicillin-intermediate and -resistant pneumococci (11, 12). Quinolones such as ciprofloxacin and ofloxacin yield moderate in vitro activity against pneumococci, with MICs clustering around the breakpoints (30, 33, 34). Newer quinolones, such as levofloxacin, gatifloxacin, moxifloxacin, and gemifloxacin, have greater antipneumococcal activities (8, 9, 14, 16, 19, 26).

Several recent reports from Hong Kong (13), Canada (7), and Spain (22) have described a worrisome increase in the incidence of quinolone-resistant pneumococci. With the increasing use of broad-spectrum quinolones that are active against pneumococci for empirical therapy of community-acquired respiratory tract infections and the eventual introduction of these compounds into the pediatric community, the incidence of these strains is likely to increase. This report examined the antipneumococcal activity of DW-224a, a new experimental quinolone (Fig. 1), by (i) determining DW-224a agar dilution MICs against 353 quinolone-susceptible strains compared to those of moxifloxacin, levofloxacin, ciprofloxacin, gatifloxacin, gemifloxacin, amoxicillin-clavulanate, cefuroxime, and azithromycin; (ii) determining, by the agar dilution method, the MICs for 29 quinolone-resistant strains with defined quinolone resistance-determining regions (QRDRs) compared to those of ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, clinafloxacin, and gemifloxacin and determining the presence of an efflux mechanism; (iii) performing time-kill studies of DW-224a, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, gemifloxacin, amoxicillin-clavulanate, cefuroxime, and azithromycin against nine selected pneumococcal strains; (iv) performing multistep studies to test the capabilities of DW-224a, moxifloxacin, levofloxacin, ciprofloxacin, gatifloxacin, gemifloxacin, amoxicillin-clavulanate, cefuroxime, and azithromycin to select for resistant

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, P.O. Box 850, Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-7953. E-mail: pappelbaum@psu.edu.

FIG. 1. DW-224a chemical structure.

mutants of nine *Streptococcus pneumoniae* strains; and (v) determining resistance mechanisms in all resistant strains and selected resistant multistep clones.

MATERIALS AND METHODS

Bacteria. For agar dilution MIC studies, 353 quinolone-susceptible pneumococci (levofloxacin MICs, ≤ 2.0 $\mu\text{g/ml}$) comprised 147 penicillin-susceptible (MICs, ≤ 0.06 $\mu\text{g/ml}$), 106 penicillin-intermediate (MICs, 0.125 to 1.0 $\mu\text{g/ml}$), and 100 penicillin-resistant (MICs, 2.0 to 16.0 $\mu\text{g/ml}$) strains. Of these, 209 were azithromycin-susceptible (MICs, ≤ 0.5), 20 were azithromycin-intermediate (MICs, 1.0 $\mu\text{g/ml}$) and 124 were azithromycin-resistant (MICs, ≥ 2.0 $\mu\text{g/ml}$) strains. Additionally, 29 strains with levofloxacin MICs of ≥ 8.0 $\mu\text{g/ml}$ from our collection (1998 to 2002) were tested by agar dilution. Nine strains were analyzed by time-kill methodology. Of these, six were quinolone susceptible and three were quinolone resistant; three strains were macrolide resistant and were not tested for azithromycin kill kinetics. For multistep resistance studies, three penicillin G-susceptible, three intermediate, and three penicillin-resistant strains (including three quinolone-resistant, two macrolide-susceptible, and six macrolide-resistant strains [two *erm*(B), two *mef*(A), one *erm*(B) plus *mef*(A), and one with altered 23S rRNA]) were tested.

Antimicrobials and MIC testing. DW-224a susceptibility powder was obtained from Dong Wha Pharmaceuticals, Anyang City, South Korea. Other antimicrobials were obtained from their respective manufacturers, and cinafloxacin was a gift from Pfizer Laboratories (Ann Arbor, MI). Amoxicillin was chosen to represent the β -lactam with the greatest potency against pneumococci with raised penicillin G MICs, and cefuroxime was chosen as a representative of oral cephalosporins with good activity against penicillin-susceptible and reasonable activity against penicillin-intermediate (but not-resistant) strains. Azithromycin represented a drug of the macrolide-lincosamide-streptogramin B group that is widely used therapeutically for the treatment of community-acquired respiratory tract infections (16, 17). Agar dilution methodology was performed using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% sheep blood (16, 17). A bacterial inoculum of 10^4 CFU/spot was used for all 353 strains. Standard quality control strains, including *S. pneumoniae* ATCC 49619 (27), were included in each run of agar dilution MICs.

Time-kill testing. Time-kill studies of nine strains were performed as described previously (10, 23, 29). Strains with macrolide MICs of only ≤ 8.0 $\mu\text{g/ml}$ were chosen for macrolide time-kill testing because of problems in solubilization at high concentrations and lack of clinical significance.

Multistep mutation analysis. Multistep resistance selection techniques were based on methods described previously by our group (9, 21, 23). Serial passages were performed daily for each of the nine tested strains in subinhibitory concentrations of all antimicrobials. For each subsequent daily passage, a 10^{-8} inoculum of culture containing ca. 10^8 CFU/ml was taken from the tube two dilutions below the MIC. Daily passages were continued until a significant (at least fourfold) increase in the MIC was found. A minimum of 14 passages was performed in every case. The maximal number of daily passages was 50. The stability of the acquired resistance was determined by MIC determination after 10 daily passages of the selected clone on drug-free agar. The four macrolide-resistant strains [two with *erm*(B), one with *erm*(B) plus *mef*(A), and one with a mutation in 23S rRNA] with azithromycin MICs of >64 $\mu\text{g/ml}$ were not tested. The identities of parents and resistant clones were confirmed throughout the study by pulsed-field gel electrophoresis (21, 23).

Determination of quinolone resistance mechanism. PCR was used to amplify fragments of *parC*, *parE*, *gyrA*, and *gyrB* genes, using primers and cycling conditions described previously (28). After amplification, PCR products were purified from excess primers and nucleotides using a QIAquick PCR purification kit as recommended by the manufacturer (QIAGEN, Valencia, CA) and sequenced directly using a CEQ8000 genetic analysis system (Beckman Coulter, Inc., Fullerton, Calif.). All clones resistant to azithromycin were tested for the presence of mutations in ribosomal proteins L4 and L22 and in domains II and V of 23S rRNA, using primers and conditions described previously (32).

Presence of quinolone efflux mechanism. The efflux mechanism was tested for all quinolone-resistant strains. MICs were determined in the presence and absence of 10 $\mu\text{g/ml}$ of reserpine (Sigma Chemicals, St. Louis, MO) as described previously (4, 9, 28). The efflux mechanism was defined as an at-least-fourfold lower MIC in the presence of reserpine.

TABLE 1. Agar dilution MICs ($\mu\text{g/ml}$) of 353 quinolone-susceptible strains^a

Drug	MIC range	MIC ₅₀	MIC ₉₀
Penicillin			
Penicillin S	≤ 0.008 –0.06	0.016	0.03
Penicillin I	0.12–1.0	0.25	1.0
Penicillin R	2.0–16	2.0	4.0
DW-224a			
Penicillin S	≤ 0.001 –0.06	0.016	0.03
Penicillin I	0.004–0.06	0.016	0.03
Penicillin R	0.004–0.03	0.016	0.03
Ciprofloxacin			
Penicillin S	0.25–4.0	1.0	2.0
Penicillin I	0.5–4.0	1.0	2.0
Penicillin R	0.25–4.0	1.0	2.0
Levofloxacin			
Penicillin S	0.12–2.0	1.0	2.0
Penicillin I	0.5–2.0	1.0	1.0
Penicillin R	0.5–2.0	1.0	1.0
Gatifloxacin			
Penicillin S	0.12–0.5	0.25	0.5
Penicillin I	0.12–0.5	0.25	0.5
Penicillin R	0.06–0.5	0.25	0.5
Moxifloxacin			
Penicillin S	0.03–0.25	0.12	0.25
Penicillin I	0.03–0.25	0.12	0.25
Penicillin R	0.03–0.25	0.12	0.25
Gemifloxacin			
Penicillin S	≤ 0.001 –0.06	0.03	0.03
Penicillin I	0.008–0.06	0.03	0.03
Penicillin R	0.008–0.06	0.03	0.03
Amoxicillin-clavulanate			
Penicillin S	≤ 0.008 –0.12	0.016	0.03
Penicillin I	0.016–2.0	0.12	1.0
Penicillin R	0.5–8.0	2.0	4.0
Cefuroxime			
Penicillin S	≤ 0.008 –0.12	0.03	0.06
Penicillin I	0.06–8.0	0.5	4.0
Penicillin R	1.0–32	4.0	16
Azithromycin			
Penicillin S	0.016– >16	0.06	>16
Penicillin I	0.03– >16	0.06	>16
Penicillin R	0.03– >16	>16	>16

^a Levofloxacin MICs, ≤ 2.0 $\mu\text{g/ml}$. S, susceptible; I, intermediate; R, resistant.

TABLE 2. Quinolone agar dilution MICs ($\mu\text{g/ml}$) of 29 quinolone-resistant strains^a

Quinolone	MIC range	MIC ₅₀	MIC ₉₀
DW-224a	0.06–1	0.12	0.25
Ciprofloxacin	8–>32	16	>32
Levofloxacin	8–32	8	16
Gatifloxacin	4–16	4	8
Moxifloxacin	2–4	2	4
Clinafloxacin	0.5–4	1	1
Gemifloxacin	0.12–1	0.25	0.25

^a Levofloxacin MICs, $\geq 8 \mu\text{g/ml}$.

RESULTS

The results of MIC testing against quinolone-susceptible pneumococci can be seen in Table 1. As shown, DW-224a was the most potent quinolone (MIC₅₀, 0.016 $\mu\text{g/ml}$; MIC₉₀, 0.03 $\mu\text{g/ml}$), followed by gemifloxacin (MIC₅₀ and MIC₉₀, 0.03 $\mu\text{g/ml}$), moxifloxacin (MIC₅₀, 0.125 $\mu\text{g/ml}$; MIC₉₀, 0.25 $\mu\text{g/ml}$), gatifloxacin (MIC₅₀, 0.25 $\mu\text{g/ml}$; MIC₉₀, 0.5 $\mu\text{g/ml}$), and levofloxacin and ciprofloxacin (MIC₅₀, 1.0 $\mu\text{g/ml}$; MIC₉₀, 2.0 $\mu\text{g/ml}$). β -Lactam MICs rose with those of penicillin G. Strains were selected such that azithromycin resistance occurred in penicillin-susceptible, -intermediate, and -resistant strains but at greater frequencies in the last two groups.

Against 29 clinical isolates that were resistant to quinolones (levofloxacin MICs, $\geq 8.0 \mu\text{g/ml}$), DW-224a also had the lowest

MICs (0.06 to 1.0 $\mu\text{g/ml}$; MIC₉₀, 0.25 $\mu\text{g/ml}$), followed by gemifloxacin (0.125 to 1 $\mu\text{g/ml}$; MIC₉₀, 0.25 $\mu\text{g/ml}$), clinafloxacin (0.5 to 4 $\mu\text{g/ml}$; MIC₉₀, 1 $\mu\text{g/ml}$), moxifloxacin (2 to 4 $\mu\text{g/ml}$; MIC₉₀, 4 $\mu\text{g/ml}$), gatifloxacin (4 to 16 $\mu\text{g/ml}$; MIC₉₀, 8 $\mu\text{g/ml}$), levofloxacin (8 to 32 $\mu\text{g/ml}$; MIC₉₀, 16 $\mu\text{g/ml}$), and ciprofloxacin (8 to >32 $\mu\text{g/ml}$; MIC₉₀, >32 $\mu\text{g/ml}$) (Table 2).

Mutations in the QRDR for the 29 strains are presented in Table 3. As can be seen, quinolone resistance was associated with mutations in the QRDR of *parC*, *gyrA*, *parE*, and/or *gyrB* genes. In 27 isolates, quinolone nonsusceptibility was associated with amino acid substitutions at position Ser79, Asp83, or Lys137 in ParC (Table 3). Most of the strains had single substitution mutations of Ser79 to Phe or Asp83 to Asn (13 strains) or double mutations of Ser79 to Phe and Lys137 to Asn (14 strains). Of 29 isolates, 27 had mutations in GyrA at amino acid position Ser81 substituted by Phe (17 strains), Tyr (8 strains), Ala (1 strain), or Cys (1 strain). Mutations in ParE were found in 22 isolates, with an amino acid substitution of Ile by Val or Asn at position 460 or a mutation of Asp435 to Asn. Mutations in GyrB were less common; only one isolate had a Glu474-to-Lys substitution (Table 3).

In the presence of reserpine, 5 of 29 strains had lower ciprofloxacin MICs (four to eight times), 2 strains had lower gemifloxacin MICs (four times), and 1 strain had lower gatifloxacin and clinafloxacin MICs (four times). Two of the strains tested had lower DW-224a MICs in the presence of reserpine.

TABLE 3. Quinolone MICs for 29 strains with defined mutations in the QRDR^a

Strain no.	MIC ($\mu\text{g/ml}$)							Detected mutation(s) in QRDRs ^c			
	DW-224a	Cipro	Levo	Moxi	Gati	Clina	Gemi	GyrA	GyrB	ParC	ParE
1055	0.12	16	16	4	4	1	0.25	S81F	—	S79F, K137N	I460V
1059	0.06	16	16	2	4	0.5	0.12	S81F	—	S79F, K137N	I460N
1060	0.06	16	8	2	4	0.5	0.12	S81F	—	S79F, K137N	I460V
1062	0.5 ^b	>32	32	4	16	4	0.5	S81F	—	S79F	I460V
1066	0.25	32	16	4	8	1	0.25	S81F	—	S79F	I460V
1068	0.25	32	8	4	8 ^b	1	0.25	—	—	S79Y, K137N	I460V
1070	0.25	16	16	4	4	1	0.25	S81F	—	D83N	I460V
1071	0.25	16	16	4	8	1	0.25	S81F	—	S79F, K137N	—
1072	0.25	>32	16	4	8	1	0.25	S81F	—	S79Y	I460V
1073	0.06	16	8	2	4	0.5	0.12	S81F	—	—	I460V
1074	0.25	32 ^b	16	4	4	1	0.25	S81F	E474K	S79F, K137N	—
1076	0.25	32 ^b	8	2	4	1	0.25	S81F	—	D83N	I460V
1077	0.12	>32	16	4	8	1	0.25	S81Y	—	S79F	—
1078	0.25	>32	16	4	8	1	0.5	S81F	—	S79F	I460V
1135	0.25	32 ^b	8	2	8	1	0.25	S81A	—	S79Y	—
1139	0.12	16	8	4	8	0.5	0.25	S81F	—	S79F, K137N	I460V
1146	0.25	32 ^b	8	2	4	1 ^b	0.25	S81F	—	—	D435N
1147	0.5 ^b	>32	8	2	4	1	0.25	S81F	—	S79F	I460V
1150	0.06	16	8	2	4	0.5	0.25	S81C	—	S79F	I460V
1156	0.25	16	8	4	8	0.5	0.25	S81F	—	S79F	—
2065	1	>32	32	4	8	1	1	—	—	S79F, K137N	—
2527	0.06	16	8	4	8	0.5	0.12	S81Y	—	S79F, K137N	I460V
2529	0.12	32 ^b	16	2	4	1	0.12	S81Y	—	S79F	I460V
2536	0.06	8	8	2	4	0.5	0.12	S81Y	—	S79F, K137N	I460V
2538	0.06	8	8	2	4	0.5	0.12	S81Y	—	S79F, K137N	I460V
2542	0.06	8	8	2	8	0.5	0.12	S81Y	—	S79F, K137N	I460V
2578	0.06	8	8	2	8	0.5	0.12	S81Y	—	S79F, K137N	I460V
4026	0.12	32	16	2	8	1	0.25 ^b	S81Y	—	S79F, K137N	I460V
5041	0.12	16	8	2	4	0.5	0.25 ^b	S81F	—	S79F	—

^a Abbreviations: Cipro, ciprofloxacin; Levo, levofloxacin; Moxi, moxifloxacin; Gati, gatifloxacin; Clina, clinafloxacin; Gemi, gemifloxacin.^b Strains for which the MICs are dropped at least fourfold in the presence of reserpine are shown.^c —, mutations not detected.

TABLE 4. MICs ($\mu\text{g/ml}$) of nine strains tested by time-kill

Drug	MIC for strain no.								
	1	2	3	4	5	6	7	8	9
DW-224a	0.016	0.03	0.25	0.25	0.016	0.03	0.125	0.008	0.016
Ciprofloxacin	1.0	2.0	32.0	16.0	1.0	2.0	16.0	0.5	1.0
Levofloxacin	0.5	1.0	16.0	16.0	1.0	1.0	16.0	1.0	1.0
Gatifloxacin	0.25	0.5	4.0	4.0	0.25	0.25	8.0	0.25	0.25
Moxifloxacin	0.125	0.125	2.0	2.0	0.125	0.125	2.0	0.125	0.125
Gemifloxacin	0.016	0.03	0.25	0.5	0.016	0.03	0.25	0.016	0.016
Amoxicillin-clavulanate	0.016	0.016	0.03	0.125	0.06	0.5	2.0	2.0	2.0
Cefuroxime	0.016	0.016	0.03	0.25	0.5	0.5	2.0	8.0	8.0
Azithromycin	>16.0	0.03	0.06	0.06	0.06	0.06	>16.0	>16.0	0.125

Moxifloxacin and levofloxacin were not efflux substrates in the 29 strains tested (Table 3).

MICs of the nine strains tested by time-kill are presented in Table 4, and kill kinetics results are presented in Table 5. As can be seen at $2\times$ MIC, DW-224a was bactericidal against eight of nine strains tested and gemifloxacin was bactericidal against seven of nine strains tested at $2\times$ MIC after 24 h. Other quinolones gave similar kill kinetics relative to higher MICs. Amoxicillin-clavulanate and cefuroxime were bactericidal against all nine strains tested at $2\times$ MIC after 24 h, and azithromycin was bactericidal against four of the six strains tested at $2\times$ MIC after 24 h.

The results of multistep resistance selection studies are presented in Table 6. DW-224a yielded resistant clones in six of nine strains after 14 to 32 days, with MIC increases from 0.008 to 0.125 $\mu\text{g/ml}$ in parents to 0.06 to 2 $\mu\text{g/ml}$ in mutants. By comparison, moxifloxacin yielded clones with raised MICs from 0.125 to 1 $\mu\text{g/ml}$ (parents) to 1 to 8 $\mu\text{g/ml}$ (resistant clones) after 19 to 50 days in seven of nine strains; levofloxacin MICs rose from 1 $\mu\text{g/ml}$ (parents) to 16 $\mu\text{g/ml}$ after 18 to 31 days in two strains; ciprofloxacin MICs rose from 2 to 16 (parents) to 16 to 128 (mutants) after 28 to 33 days in two strains; gatifloxacin MICs rose from 1 to 2 $\mu\text{g/ml}$ to 16 $\mu\text{g/ml}$ after 14 to 20 days in two strains; and gemifloxacin MICs rose from 0.016 to 0.5 (parents) to 0.125 to 8 (mutants) in five strains after 24 to 42 days. Azithromycin selected for resistant clones in three of the five parents tested after 20 to 33 days, with MICs increasing from 0.016 to 0.03 $\mu\text{g/ml}$ (parents) to 0.25 to >64 $\mu\text{g/ml}$ (resistant clones). Amoxicillin-clavulanate and cefuroxime did not yield resistant clones after 50 days.

Cross-resistance activity was observed among quinolones. A total of 15 clones showed cross-reactivity; 2 clones with raised DW-224a MICs showed cross-reaction to four other quinolones, and 12 clones with raised quinolone MICs had cross-reaction to DW-224a (Table 6).

Changes in the QRDR were common in all quinolone-selected mutants, but DW-224a MICs in quinolone-resistant clones selected by DW-224a were lower than those of resistant strains with the same mutations but selected by other quinolones. A change in ParE (Pro454 to Ser) was present only in mutants selected by moxifloxacin (Table 6). Mutations in 23S rRNA or L22 were present in two out of three selected azithromycin-resistant clones (Table 6).

TABLE 5. Results of kill kinetics studies

Drug	No. of strains killed ^a											
	3 h			6 h			12 h			24 h		
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3
DW-224a												
4 $\times$ MIC	8	1	0	9	4	1	9	9	4	9	9	8
2 $\times$ MIC	7	0	0	9	1	1	9	9	2	9	9	8
MIC	3	0	0	7	1	0	9	4	1	9	8	4
Ciprofloxacin												
4 $\times$ MIC	7	1	1	8	3	1	9	8	2	9	9	8
2 $\times$ MIC	5	1	0	7	1	1	9	6	1	9	9	7
MIC	1	0	0	7	1	0	9	4	1	9	7	3
Levofloxacin												
4 $\times$ MIC	6	1	1	9	5	1	9	9	3	9	9	8
2 $\times$ MIC	6	1	0	9	1	1	9	9	2	9	9	7
MIC	3	0	0	7	1	1	8	6	1	8	6	5
Gatifloxacin												
4 $\times$ MIC	8	1	0	9	3	1	9	9	3	9	9	8
2 $\times$ MIC	7	1	0	9	1	1	9	9	2	9	9	8
MIC	3	0	0	7	0	0	7	5	1	7	6	5
Moxifloxacin												
4 $\times$ MIC	8	1	0	9	2	1	9	9	4	9	9	8
2 $\times$ MIC	5	0	0	9	1	1	9	8	2	9	9	8
MIC	2	0	0	5	1	0	9	3	1	9	6	3
Gemifloxacin												
4 $\times$ MIC	7	1	0	9	2	1	9	9	3	8	8	7
2 $\times$ MIC	6	1	0	8	1	1	9	8	3	8	8	7
MIC	3	0	0	5	1	0	8	5	1	7	4	2
Amoxicillin-clavulanate												
4 $\times$ MIC	9	4	1	9	6	3	9	9	7	9	9	9
2 $\times$ MIC	9	3	0	9	6	3	9	9	7	9	9	9
MIC	9	2	0	8	5	1	8	8	6	7	7	6
Cefuroxime												
4 $\times$ MIC	8	1	0	9	4	2	9	9	3	9	9	9
2 $\times$ MIC	7	1	0	8	4	1	9	9	3	9	9	9
MIC	6	0	0	8	2	0	9	6	1	7	7	4
Azithromycin ^b												
4 $\times$ MIC	3	0	0	4	0	0	6	3	0	6	6	5
2 $\times$ MIC	1	0	0	4	0	0	5	2	0	6	5	4
MIC	0	0	0	2	0	0	5	0	0	6	3	2

^a -1, 90% killing; -2, 99% killing; -3, 99.9% killing.

^b Three strains with azithromycin MICs of >16.0 $\mu\text{g/ml}$ were not tested by time-kill.

3243	DW-224a	0.03	0.25	28	0.125	0.125	0.5	0.03	0.25	1	0.06	1	4	—
	Amox/clav	0.06	0.25	50										NT
	Cefuroxime	0.25	1	50										NT
	Azithro	0.03	0.25	33	≤0.016	0.125	0.5	0.5	0.25	0.5	0.03	0.25	0.5	L22 insertion: 93SFRPRA94
	Moxi	0.125	1	20	0.03	0.06	0.25	0.016	1	1	0.03	1	2	—
	Levo	0.5	2	50										NT
	Cipro	1	4	50										NT
	Gati	0.5	0.5	50										NT
	Gemi	0.016	0.06	50										NT
	DW-224a	0.016	0.03	50										NT
19	Amox/clav	2	4	50										NT
	Cefuroxime	2	4	50										NT
	Azithro	>128	NT	NT										NT
	Moxi	0.125	1	32	0.06	1	2	>128	2	2	0.06	0.5	1	GyrA mutation: S81F
	Levo	1	16	31	0.125	1	2	>128	4	32	0.125	4	16	Mutations: GyrA (S81Y), ParC (D83Y)
	Cipro	1	2	50										NT
	Gati	0.25	0.5	50										NT
	Gemi	0.016	0.03	50										NT
	DW-224a	0.016	0.06	50										NT
	Amox/clav	1	2	50										NT
24	Cefuroxime	2	4	50										NT
	Azithro	>128	NT	NT										NT
	Moxi	0.25	4	50	0.25	1	2	>128	4	32	0.25	4	32	Mutations: GyrA (S81Y), GyrB (P413S), ParC (S79F)
	Levo	1	2	50										NT
	Cipro	2	16	28	0.125	1	2	>128	8	32	0.25	8	16	Mutations: GyrA (S81Y), ParC (S79Y)
	Gati	0.5	0.5	50										NT
	Gemi	0.016	0.5	26	0.5	1	2	>128	8	32	0.5	8	32	Mutations: GyrA (S81Y), ParC (S79Y)
	DW-224a	0.06	0.5	32	0.5	2	16	0.03	8	64	1	16	32	Mutations: GyrB (S405F), ParC (D83N)
	Amox/clav	1	2	50										NT
	Cefuroxime	8	32	50										NT
2527	Azithro	0.016	4	20	0.125	2	16	4	4	32	0.25	8	32	—
	Moxi	2	8	50										NT
	Levo	16	32	50										NT
	Cipro	16	128	33	0.5	2	16	0.03	8	32	1	16	64	—
	Gati	4	8	50										NT
	Gemi	0.25	1	50										NT
	DW-224a	0.06	0.5	32	0.5	2	16	0.03	8	64	1	16	32	Mutations: GyrB (S405F), ParC (D83N)
	Amox/clav	1	2	50										NT
	Cefuroxime	8	32	50										NT
	Azithro	0.016	4	20	0.125	2	16	4	4	32	0.25	8	32	—

^a Abbreviations: Amox/clav, amoxicillin-clavulanate; Azithro, azithromycin; Moxi, moxifloxacin; Levo, levofloxacin; Gemi, gemifloxacin; Cipro, ciprofloxacin; NT, mechanism not tested.

^b Cross-resistance strains are depicted in bold.

^c —, resistance mechanism not detected.

^d *Escherichia coli* numbering.

DISCUSSION

DW-224a is a new broad-spectrum quinolone with potent activity against respiratory bacteria, including *S. pneumoniae* (J. Kwak, M. J. Seol, H. J. Kim, H. S. Park, D. R. Choi, and Y. H. Yung, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-415, 2003; H. J. Kwon, S. K. Ku, D. H. Jeong, S. H. Hwang, J. M. Ryu, Y. H. Jung, M. Lee, J. H. Lee, and M. K. Chung, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-417, 2003). MICs obtained in the current study are similar to those found in two preliminary studies.

In our study, DW-224a gave the lowest quinolone MICs against all pneumococcal strains tested, followed by gemifloxacin, moxifloxacin, gatifloxacin, levofloxacin, and ciprofloxacin. MICs of all quinolones were similar to those described previously (8, 9, 14, 19, 26, 30, 33, 34). Additionally, DW-224a gave significantly lower MICs against highly quinolone-resistant pneumococci, irrespective of the quinolone resistance mechanism. This was the case for double mutants with mutations in both *parC* and *gyrA*, strains which have previously been shown to be highly resistant to other quinolones, as well as for strains with an efflux mechanism. MICs of nonquinolone agents were similar to those described previously, with higher β -lactam and macrolide MICs in strains with raised penicillin MICs (8, 9, 14, 31, 33, 34).

The contribution of the efflux mechanism to higher MICs of quinolones has been found to be important only for ciprofloxacin (4). Gemifloxacin, moxifloxacin, gatifloxacin, and levofloxacin are less affected by the efflux mechanism as is the new fluoroquinolone DW-224a in the current study (8, 9, 26).

Results of multistep resistance selection studies showed that DW-224a gave the lowest initial MICs and MICs of quinolone-resistant mutants, even in quinolone-resistant parents, with parent and mutant MICs several dilutions lower than those obtained with other quinolones. Cross-resistance between quinolone-selected clones and mutants with raised DW-224a MICs may represent the same targeting of resistant determinates characteristic for this group of antimicrobials.

Alterations in the QRDR were detected among resistant mutants selected by quinolone exposure. In *S. pneumoniae*, some fluoroquinolones appear to target preferentially DNA topoisomerase IV or DNA gyrase, but some of them (gemifloxacin and moxifloxacin) have been assumed to target both (20). Only moxifloxacin exposure selected mutations in all four QRDRs. DW-224a-selected mutations in *GyrA*, *GyrB*, and *ParC* and the other four quinolones selected alterations only in *GyrA* and *ParC* proteins. All of these mutations in any QRDR are known to be involved in high-level resistance to quinolones and have been reported previously (5, 18, 21). Only three alterations in *GyrB*, Ser478 to Ile, Glu474 to Lys, and Pro413 to Ser, have, to our knowledge, not yet been described. Among all quinolones tested, DW-224a yielded clones with the lowest MICs. Changes in 23S rRNA and L22 protein were detected in two out of three resistant clones selected by azithromycin. Mutations in 23S rRNA (A2058G) and alteration in L22 protein have been described previously, and a correlation between the presence of these mutations and resistance development has been shown (6, 23).

Kill kinetic studies showed that all quinolones gave similar

cidalities relative to their MICs. When MICs and kill kinetics were taken together, however, DW-224a and gemifloxacin gave the best activity against all groups of pneumococcal strains, irrespective of their drug susceptibility.

In summary, DW-224a was the most potent quinolone tested against both quinolone-susceptible and -resistant pneumococci. The incidence of quinolone-resistant pneumococci is currently very low. However, this situation may change with the introduction of broad-spectrum quinolones into clinical practice, in particular in the pediatric population, leading to the selection of quinolone-resistant strains. DW-224a is a promising new antipneumococcal agent, irrespective of the susceptibility of pneumococci to quinolones and other agents. Pharmacokinetic and pharmacodynamic studies (free area under the concentration-time curve/MIC or maximum concentration of drug in serum/MIC), followed by toxicity and animal studies, are required to see whether this compound will be promising clinically.

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