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In Vitro Activity of the Novel Lactone Ketolide Nafithromycin (WCK 4873) against Contemporary Clinical Bacteria from a Global Surveillance Program

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ABSTRACT Nafithromycin (WCK 4873), a novel antimicrobial agent of the lactone ketolide class, is currently in phase 2 development for treatment of community-acquired bacterial pneumonia (CABP). A total of 4,739 nonduplicate isolates were selected from a 2014 global surveillance program at medical institutions located in 43 countries within the United States, Europe, Latin America, and the Asia-Pacific region. Nafithromycin and comparator agents were used for susceptibility testing by reference broth microdilution methods. Nafithromycin was active against *Staphylococcus aureus* ($MIC_{50/90}$, 0.06/>2 μg/ml), including erythromycin-resistant strains exhibiting an inducible clindamycin resistance phenotype ($MIC_{50/90}$, 0.06/0.06 μg/ml) and telithromycin-susceptible strains ($MIC_{50/90}$, 0.06/0.06 μg/ml), but it exhibited limited activity against most telithromycin-resistant and clindamycin-resistant isolates that were constitutively resistant to macrolides ($MIC_{50/90}$, >2/>2 μg/ml). Nafithromycin was very active ($MIC_{50/90}$, 0.015/0.06 μg/ml) against 1,911 *Streptococcus pneumoniae* strains, inhibiting all strains, with MIC values of ≤0.25 μg/ml. Telithromycin susceptibility was 99.9% for *Streptococcus pneumoniae* strains, and nafithromycin was up to 8-fold more potent than telithromycin. Overall, 37.9% of *S. pneumoniae* strains were resistant to erythromycin, and 19.7% were resistant to clindamycin. Nafithromycin was highly active against 606 *Streptococcus pyogenes* strains ($MIC_{50/90}$, 0.015/0.015 μg/ml), inhibiting 100.0% of isolates at ≤0.5 μg/ml, and $MIC_{50/90}$ values (0.015/0.015 to 0.03 μg/ml) were similar for the 4 geographic regions. Nafithromycin and telithromycin demonstrated comparable *in vitro* activities against 1,002 *Haemophilus influenzae* isolates and 504 *Moraxella catarrhalis* isolates. Overall, nafithromycin showed potent *in vitro* activity against a broad range of contemporary (2014) global pathogens. These results support the continued clinical development of nafithromycin for treatment of CABP.

KEYWORDS *Streptococcus pneumoniae*, *Streptococcus pyogenes*, ketolides

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Nafithromycin (WCK 4873) is a novel antimicrobial agent of the lactone ketolide class that was awarded qualified infectious disease product (QIDP) status by the U.S. Food and Drug Administration (U.S. FDA) in 2015. Nafithromycin has been shown to exhibit potent *in vitro* activity against respiratory tract pathogens, including multidrug-resistant *Streptococcus pneumoniae* (1, 2). It is currently undergoing clinical development in the United States that includes a pharmacokinetic study (ClinicalTrials.gov identifier NCT02770404) and a phase 2 study for the treatment of community-acquired bacterial pneumonia (CABP) (ClinicalTrials.gov identifier NCT02903836). In Europe, it has completed single ascending dose (SAD) and multiple ascending dose (MAD) phase 1 pharmacokinetic studies (3, 4).

Ketolides are derivatives of the erythromycin A class that lack the L-cladinose sugar at position 3 of the erythronolide ring and have a ketone in its place (5). Ketolides

interact at multiple positions on the ribosome, thus allowing for activity against macrolide-resistant organisms even though the presence of ribosome methylation prevents macrolides from being active (5–8). Due to a spectrum of activity that includes macrolide-resistant organisms, the ketolides have been viewed as an attractive choice for treating infections where multidrug-resistant Gram-positive bacteria may occur, such as CABP (5, 9, 10).

In this study, we evaluated the activities of nafithromycin and comparator antimicrobial agents, measured by reference Clinical and Laboratory Standards Institute (CLSI) methods, and tested them against a large collection of contemporary clinical isolates collected in medical centers worldwide as part of the 2014 SENTRY Antimicrobial Surveillance Program.

RESULTS

Nafithromycin demonstrated bimodal activity ($MIC_{50/90}$, 0.06/>2 μg/ml) against 716 *Staphylococcus aureus* isolates (Tables 1 and 2). Regardless of the methicillin-resistant *S. aureus* (MRSA) profile, nafithromycin showed limited activity (MICs of >2 μg/ml) against most (80/83 strains) telithromycin-resistant ($n = 80$) and telithromycin-intermediate ($n = 3$) strains that were also clindamycin resistant, i.e., constitutively resistant to macrolides (Tables 1 and 2). Conversely, nafithromycin activity was high ($MIC_{50/90}$, 0.06/0.06 μg/ml) against telithromycin-susceptible strains as well as against erythromycin-resistant strains that were susceptible to clindamycin ($n = 85$) and strains with inducible clindamycin resistance (Table 1). The activity of nafithromycin varied by geographic region, with activity being greater in Europe (EU) ($MIC_{50/90}$, 0.06/0.06 μg/ml) (Table 2) and the Asia-Pacific region (APAC) ($MIC_{50/90}$, 0.06/0.12 μg/ml) (Table 2) than in the United States (US) ($MIC_{50/90}$, 0.06/>2 μg/ml) (Table 2) and Latin America (LATAM) ($MIC_{50/90}$, 0.06/>2 μg/ml) (Table 2). However, the overall MIC distributions were similar (bimodal) for these regions, and the lower MIC_{90} values observed for the EU and APAC were reflective of the lower rates of telithromycin resistance in these regions (6.9% and 8.4%, respectively) than in the US (12.2%) and LATAM (18.2%) (Table 2).

Against *S. aureus* isolates from all geographic regions combined, nafithromycin ($MIC_{50/90}$, 0.06/>2 μg/ml) demonstrated a potency most similar to that of telithromycin ($MIC_{50/90}$, 0.06/>2 μg/ml) (88.4% susceptible). Overall, resistance rates were higher for oxacillin (MRSA) (34.6%), erythromycin (33.1 to 37.2% [CLSI/EUCAST]), ciprofloxacin (25.1 to 27.1% [CLSI/EUCAST]), and clindamycin (11.9% [data not shown]). MRSA rates ranged from 24.6% in the EU (Table 2) to 46.3% in the US (Table 2). Erythromycin resistance rates ranged from 24.7% in APAC (Table 2) to 56.1% in the US (Table 2). No resistance was observed when *S. aureus* isolates were tested against linezolid and vancomycin (Table 2).

Nafithromycin was very active ($MIC_{50/90}$, 0.015/0.06 μg/ml) against *S. pneumoniae* isolates, with all isolates inhibited at MIC values of 0.25 μg/ml or less (Tables 1 and 2). Similar $MIC_{50/90}$ values were observed for the 4 geographic regions sampled (Table 2). Overall, telithromycin susceptibility was 99.9%/89.3% (CLSI/EUCAST criteria [data not shown]). Nafithromycin (MIC_{90}, 0.06 μg/ml) was 8-fold more potent than telithromycin (MIC_{90}, 0.5 μg/ml) against *S. pneumoniae*. Against erythromycin- and clindamycin-resistant pneumococci (constitutively macrolide resistant), the MIC_{50} and MIC_{90} of nafithromycin were 0.03 and 0.12 μg/ml, respectively (Tables 1 and 2). Importantly, nafithromycin was very active against telithromycin-nonsusceptible *S. pneumoniae* isolates, with MIC_{50} and MIC_{90} values of 0.06 and 0.12 μg/ml, respectively (Tables 1 and 2).

Using the EUCAST susceptibility breakpoint of ≤0.25 μg/ml for telithromycin, 204 *S. pneumoniae* isolates were nonsusceptible to telithromycin, and all of these strains demonstrated nafithromycin MIC values of ≤0.25 μg/ml ($MIC_{50/90}$, 0.06/0.12 μg/ml) (Table 1). Nafithromycin retained good activity against erythromycin-resistant/clindamycin-susceptible ($MIC_{50/90}$, 0.03/0.06 μg/ml) and erythromycin-resistant/clindamycin-resistant ($MIC_{50/90}$, 0.03/0.12 μg/ml) (Table 1) isolates. Using CLSI breakpoints, resistance to oral

TABLE 1 Cumulative frequency distributions of nafithromycin MIC results against 4,739 bacterial isolates (all regions combined)

Organism or phenotype (MIC)[c]	No. of isolates	No. of isolates (cumulative %) with nafithromycin MIC (μg/ml)															MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
		≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>[a]		
S. aureus	716				1 (0.1)	39 (5.6)	562 (84.1)	27 (87.8)	3 (88.3)	2 (88.5)	0 (88.5)	2 (88.8)				80 (100.0)	0.06	>2
MSSA	468					28 (6.0)	409 (93.4)	20 (97.6)	1 (97.9)	2 (98.3)	0 (98.3)	2 (98.7)				6 (100.0)	0.06	0.06
MRSA	248				1 (0.4)	11 (4.8)	153 (66.5)	7 (69.4)	2 (70.2)	0 (70.2)	0 (70.2)	0 (70.2)				74 (100.0)	0.06	>2
Telithromycin^{S} (≤1 μg/ml)	633				1 (0.2)	39 (6.3)	562 (95.1)	27 (99.4)	3 (99.8)	1 (100.0)							0.06	0.06
Telithromycin^{I} (2 μg/ml)	3									1 (33.3)	0 (33.3)	0 (33.3)				2 (100.0)	>2	
Telithromycin^{R} (≥4 μg/ml)	80											2 (2.5)				78 (100.0)	>2	>2
ERY^{r} (≥1 μg/ml), CLI^{s} (≤0.5 μg/ml), D test^{-b}	85					3 (3.5)	74 (90.6)	8 (100.0)									0.06	0.06
ERY^{r} (≥1 μg/ml), CLI^{s} (≤0.5 μg/ml), D test^{+b}	110					11 (10.0)	93 (94.5)	4 (98.2)	0 (98.2)	1 (99.1)	0 (99.1)	0 (99.1)				1 (100.0)	0.06	0.06
CLI^{NS} (≥1 μg/ml)	85							1 (1.2)	2 (3.5)	1 (4.7)	0 (4.7)	2 (7.1)				79 (100.0)	>2	>2
ERY^{s} (≤0.5 μg/ml), CLI^{s} (≤0.5 μg/ml)	436				1 (0.2)	25 (6.0)	395 (96.6)	14 (99.8)	1 (100.0)								0.06	0.06
S. pneumoniae	1,911	1 (0.1)	20 (1.1)	396 (21.8)	1014 (74.9)	224 (86.6)	162 (95.1)	88 (99.7)	6 (100.0)								0.015	0.06
PEN^{s} (≤2 μg/ml)	1,762	1 (0.1)	20 (1.2)	392 (23.4)	994 (79.9)	200 (91.2)	127 (98.4)	25 (99.8)	3 (100.0)								0.015	0.03
PEN^{i} (4 μg/ml)	133			4 (3.0)	18 (16.5)	21 (32.3)	29 (54.1)	59 (98.5)	2 (100.0)								0.06	0.12
PEN^{r} (≥8 μg/ml)	16				2 (12.5)	3 (31.2)	6 (68.8)	4 (93.8)	1 (100.0)								0.06	0.12
ERY^{s} (≤0.25 μg/ml), CLI^{s} (≤0.25 μg/ml)	1,173		19 (1.6)	371 (33.2)	765 (98.5)	14 (99.7)	3 (99.9)	1 (100.0)									0.015	0.015
ERY^{r} (≥0.5 μg/ml), CLI^{s} (≤0.25 μg/ml)	344	1 (0.3)	1 (0.6)	11 (3.8)	121 (39.0)	114 (72.1)	88 (97.7)	8 (100.0)									0.03	0.06
ERY^{r} (≥0.5 μg/ml), CLI^{r} (≥0.5 μg/ml)	394			14 (3.6)	128 (36.0)	96 (60.4)	71 (78.4)	79 (98.5)	6 (100.0)								0.03	0.12
Telithromycin^{S} (≤0.25 μg/ml)	1,707	1 (0.1)	20 (1.2)	396 (24.4)	1014 (83.8)	209 (96.1)	64 (99.8)	3 (100.0)									0.015	0.03
Telithromycin^{NS} (≥0.5 μg/ml)	204					15 (7.4)	98 (55.4)	85 (97.1)	6 (100.0)								0.06	0.12
S. pyogenes	606			46 (7.6)	513 (92.2)	22 (95.9)	10 (97.5)	6 (98.5)	2 (98.8)	7 (100.0)							0.015	0.015
ERY^{s} (≤0.25 μg/ml), CLI^{s} (≤0.25 μg/ml)	542			42 (7.7)	483 (96.9)	16 (99.8)	1 (100.0)										0.015	0.015
ERY^{r} (≥0.5 μg/ml), CLI^{s} (≤0.25 μg/ml)	33			3 (9.1)	18 (63.6)	4 (75.8)	7 (97.0)	1 (100.0)									0.015	0.06
ERY^{r} (≥0.5 μg/ml), CLI^{r} (≥0.5 μg/ml)	31			1 (3.2)	12 (41.9)	2 (48.4)	2 (54.8)	5 (71.0)	2 (77.4)	7 (100.0)							0.06	0.5
H. influenzae	1002							2 (0.2)	2 (0.4)	6 (1.0)	42 (5.2)	428 (47.9)	455 (93.3)	56 (98.9)	5 (99.4)	6 (100.0)	4	4
β-Lactamase positive	210										5 (2.4)	90 (45.2)	99 (92.4)	14 (99.0)	1 (99.5)	1 (100.0)	4	4
β-Lactamase negative	792							2 (0.3)	2 (0.5)	6 (1.3)	37 (5.9)	338 (48.6)	356 (93.6)	42 (98.9)	4 (99.4)	5 (100.0)	4	4
M. catarrhalis	504		1 (0.2)	6 (1.4)	1 (1.6)	6 (2.8)	58 (14.3)	265 (66.9)	159 (98.4)	8 (100.0)							0.12	0.25

[a]The MIC was greater than the highest concentration tested, which was 16 μg/ml for *H. influenzae* and 2 μg/ml for all other organisms.

[b]The D test was performed by broth microdilution as described in CLSI document M100-S26 (20).

[c]Abbreviations: MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; ^{s}, susceptible; ^{i}, intermediate; ^{r}, resistant; ERY, erythromycin; CLI, clindamycin; PEN, penicillin; ^{ns}, nonsusceptible.

TABLE 2 Activities by geographic region for nafithromycin and comparator antimicrobial agents tested against selected clinical isolates

	US					EU					LATAM					APAC				
				% susceptible/ % resistant					% susceptible/ % resistant					% susceptible/ % resistant					% susceptible/ % resistant	
Organism or antimicrobial agent	*n*	MIC_{50}[f]	MIC_{90}[f]	CLSI[a]	EUCAST[a]	*n*	MIC_{50}[f]	MIC_{90}[f]	CLSI[a]	EUCAST[a]	*n*	MIC_{50}[f]	MIC_{90}[f]	CLSI[a]	EUCAST[a]	*n*	MIC_{50}[f]	MIC_{90}[f]	CLSI[a]	EUCAST[a]
S. aureus	205					203					154					154				
Nafithromycin		0.06	>2				0.06	0.06				0.06	>2				0.06	0.12		
Telithromycin		0.12	>2	86.8/12.2			0.06	0.12	93.1/6.9			0.06	>2	81.2/18.2			0.06	0.25	91.6/8.4	
Clindamycin		≤0.25	>2	86.3/13.7	86.3/13.7		≤0.25	≤0.25	92.6/7.4	91.6/7.4		≤0.25	>2	81.2/18.8	80.5/18.8		≤0.25	≤0.25	91.6/8.4	90.9/8.4
Erythromycin		4	>16	40.5/49.3	42.0/56.1		0.25	>16	73.9/23.2	73.9/25.6		0.25	>16	57.8/36.4	57.8/39.6		0.25	>16	74.0/21.4	74.0/24.7
Linezolid		1	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0
Oxacillin		1	>2	53.7/46.3	53.7/46.3		0.5	>2	75.4/24.6	75.4/24.6		0.5	>2	63.6/36.4	63.6/36.4		0.5	>2	69.5/30.5	69.5/30.5
Trimethoprim-sulfamethoxazole		≤0.5	≤0.5	98.5/1.5	98.5/1.5		≤0.5	≤0.5	99.5/0.5	99.5/0.5		≤0.5	≤0.5	99.4/0.6	99.4/0.0		≤0.5	≤0.5	97.4/2.6	97.4/1.9
Vancomycin		1	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0
MRSA	95					50					56					47				
Nafithromycin		0.06	>2				0.06	>2				0.06	>2				0.06	>2		
Telithromycin		0.12	>2	76.8/23.2			0.12	>2	76.0/24.0			0.12	>2	51.8/46.4			0.06	>2	72.3/27.7	
Clindamycin		≤0.25	>2	76.8/23.2	76.8/23.2		≤0.25	>2	74.0/26.0	72.0/26.0		≤0.25	>2	51.8/48.2	50.0/48.2		≤0.25	>2	72.3/27.7	70.2/27.7
Erythromycin		>16	>16	18.9/70.5	20.0/78.9		>16	>16	34.0/60.0	34.0/66.0		>16	>16	37.5/62.5	37.5/62.5		>16	>16	34.0/57.4	34.0/61.7
Linezolid		1	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0
Trimethoprim-sulfamethoxazole		≤0.5	≤0.5	96.8/3.2	96.8/3.2		≤0.5	≤0.5	98.0/2.0	98.0/2.0		≤0.5	≤0.5	100.0/0.0	100.0/0.0		≤0.5	1	91.5/8.5	91.5/6.4
Vancomycin		1	1	100.0/0.0	100.0/0.0		0.5	1	100.0/0.0	100.0/0.0		1	1	100.0/0.0	100.0/0.0		1	1	100.0/00	100.0/0.0
S. pneumoniae	712					698					250					251				
Nafithromycin		0.015	0.06				0.015	0.03				0.015	0.06				0.015	0.06		
Telithromycin		0.015	0.5	99.9/0.0	82.7/2.7		0.015	0.12	99.9/0.0	94.8/1.0		0.015	0.25	100.0/0.0	90.8/3.2		0.015	0.25	100.0/0.0	91.2/0.0
Amoxicillin-clavulanate		≤1	4	88.5/7.2[b]			≤1	2	92.6/5.2[b]			≤1	8	79.2/12.8[b]			≤1	8	79.7/16.3[b]	
Ceftriaxone		≤0.06	1	81.6/6.3[c]	81.6/0.8		≤0.06	1	84.2/7.2[c]	84.2/1.0		0.12	2	69.2/13.6[c]	69.2/0.8		0.12	2	64.1/19.5[c]	64.1/4.8
				93.7/0.8[b]					92.8/1.0[b]					86.4/0.8[b]					80.5/4.8[b]	
Clindamycin		≤0.25	>2	82.2/16.6	83.4/16.6		≤0.25	>2	80.8 18.2	81.8 18.2		≤0.25	>2	78.4/21.6	78.4/21.6		≤0.25	>2	68.5 30.7	69.3 30.7
Erythromycin		0.25	>16	50.0/49.0	50.0/49.0		≤0.12	>16	74.1/25.4	74.1/25.4		≤0.12	>16	62.8/37.2	62.8/37.2		≤0.12	>16	57.0/42.2	57.0/42.2
Penicillin		≤0.06	2	58.1/13.6[d]	58.1/41.9[c]		≤0.06	2	68.5/11.9[d]	68.5/31.5[c]		0.12	4	49.2/26.8[d]	49.2/50.8[c]		≤0.06	4	51.8/29.1[d]	51.8/48.2[c]
				93.5/0.6[e]					95.0/0.7[e]					88.4/0.4[e]					84.5/2.4[e]	
Trimethoprim-sulfamethoxazole		≤0.5	>4	67.8/20.1	75.4/20.1		≤0.5	>4	70.6/20.1	76.1/20.1		1	>4	48.8/39.6	53.6/39.6		≤0.5	>4	56.6/34.7	64.5/34.7
Penicillin-resistant *S. pneumoniae*	4					5					1					6				
Nafithromycin		0.03					0.06										0.06			
Telithromycin		0.25		100.0/0.0	50.0/25.0		0.25		100.0/0.0	60.0/40.0				100.0/0.0	0.0/0.0		0.25		100.0/0.0	66.7/0.0
Amoxicillin-clavulanate		>8		0.0/100.0[b]			>8		0.0/80.0[b]					0.0/100.0[b]			>8		0.0/83.3[b]	
Ceftriaxone		8		0.0/100.0[c]	0.0/75.0		8		0.0/80.0[c]	0.0/60.0				0.0/100.0[c]	0.0/0.0		4		0.0/83.3[c]	0.0/66.7
				0.0/75.0[b]					20.0/60.0[b]					0.0/0.0[b]					16.7/66.7[b]	
Clindamycin		≤0.25		50.0/50.0	50.0/50.0		>2		20.0/80.0	20.0/80.0				0.0/100.0	0.0/100.0		>2		0.0/100.0	0.0/100.0
Erythromycin		8		0.0/100.0	0.0/100.0		>16		0.0/100.0	0.0/100.0				0.0/100.0	0.0/100.0		>16		0.0/100.0	0.0/100.0
Penicillin		8		0.0/100.0[d]	0.0/100.0[c]		8		0.0/100.0[d]	0.0/100.0[c]				0.0/100.0[d]	0.0/100.0[c]		8		0.0/100.0[d]	0.0/100.0[c]
				0.0/100.0[e]					0.0/100.0[e]					0.0/100.0[e]					0.0/100.0[e]	
Trimethoprim-sulfamethoxazole		4		0.0/100.0	0.0/100.0		>4		0.0/60.0	40.0/60.0				0.0/100.0	0.0/100.0		>4		0.0/100.0	0.0/100.0
Erythromycin-resistant and clindamycin-resistant *S. pneumoniae*	127					134					54					79				
Nafithromycin		0.03	0.12				0.03	0.12				0.03	0.12				0.03	0.12		
Telithromycin		0.06	0.5	100.0/0.0	66.9/6.3		0.03	0.5	99.3/0.0	79.9/5.2		0.12	0.5	100.0/0.0	64.8/9.3		0.06	0.5	100.0/0.0	77.2/0.0
Amoxicillin-clavulanate		≤1	8	57.5/33.9[b]			≤1	8	79.1/16.4[b]			4	8	46.3/40.7[b]			4	8	45.6/46.8[b]	
Ceftriaxone		1	2	48.8/28.3[c]	48.8/1.6		0.5	2	51.5/24.6[c]	51.5/3.7		1	2	29.6/31.5[c]	29.6/1.9		2	4	19.0/50.6[c]	19.0/12.7
				71.7/1.6[b]					75.4/3.7[b]					68.5/1.9[b]					49.4/12.7[b]	
Clindamycin		>2	>2	0.0/92.9	7.1/92.9		>2	>2	0.0/94.8	5.2/94.8		>2	>2	0.0/100.0	0.0/100.0		>2	>2	0.0/97.5	2.5/97.5
Erythromycin		>16	>16	0.0/100.0	0.0/100.0		>16	>16	0.0/100.0	0.0/100.0		>16	>16	00.0/100.0	0.0/100.0		>16	>16	0.0/98.7	0.0/98.7

(Continued on next page)

TABLE 2 (Continued)

	US					EU					LATAM					APAC				
				% susceptible/ % resistant					% susceptible/ % resistant					% susceptible/ % resistant					% susceptible/ % resistant	
Organism or antimicrobial agent	*n*	MIC_{50}[f]	MIC_{90}[f]	CLSI[a]	EUCAST[a]	*n*	MIC_{50}[f]	MIC_{90}[f]	CLSI[a]	EUCAST[a]	*n*	MIC_{50}[f]	MIC_{90}[f]	CLSI[a]	EUCAST[a]	*n*	MIC_{50}[f]	MIC_{90}[f]	CLSI[a]	EUCAST[a]
Penicillin		1	4	15.0/44.9[d] 66.9/1.6[e]	15.0/85.0[c]		0.5	4	20.9/38.1[d] 82.8/3.0[e]	20.9/79.1[c]		2	4	9.3/64.8[d] 63.0/1.9[e]	9.3/90.7[c]		2	4	6.3/68.4[d] 55.7/7.6[e]	6.3/93.7[c]
Trimethoprim-sulfamethoxazole		2	>4	37.8/49.6	47.2/49.6		1	>4	43.3/41.8	53.7/41.8		4	>4	29.6/59.3	35.2/59.3		>4	>4	25.3/64.6	32.9/64.6
Telithromycin- nonsusceptible *S. pneumoniae*	123					36					23					22				
Nafithromycin		0.06	0.12				0.12	0.12				0.12	0.12				0.12	0.12		
Telithromycin		0.5	1	99.2/0.0	0.0/15.4		0.5	1	97.2/0.0	0.0/19.4		0.5	1	100.0/0.0	0.0/34.8		0.5	0.5	100.0/0.0	0.0/0.0
Amoxicillin-clavulanate		≤1	8	61.8/26.8[b]			4	8	47.2/41.7[b]			8	8	8.7/65.2[b]			8	8	27.3/59.1[b]	
Ceftriaxone		0.5	2	54.5/23.6[c] 76.4/2.4[b]	54.5/2.4		1	2	22.2/44.4[c] 55.6/8.3[b]	22.2/8.3		2	2	8.7/60.9[c] 39.1/0.0[b]	8.7/0.0		2	2	18.2/54.5[c] 45.5/9.1[b]	18.2/9.1
Clindamycin		≤0.25	>2	65.9/31.7	68.3/31.7		>2	>2	25.0/75.0	25.0/75.0		>2	>2	17.4/82.6	17.4/82.6		>2	>2	18.2/81.8	18.2/81.8
Erythromycin		16	>16	0.0/100.0	0.0/100.0		>16	>16	0.0/100.0	0.0/100.0		>16	>16	0.0/100.0	0.0/100.0		>16	>16	0.0/100.0	0.0/100.0
Penicillin		1	4	41.5/40.7[d] 72.4/1.6[e]	41.5/58.5[c]		2	4	19.4/69.4[d] 55.6/5.6[e]	19.4/80.6[c]		4	4	0.0/87.0[d] 39.1/4.3[e]	0.0/100.0[c]		4	4	18.2/81.8[d] 40.9/9.1[e]	18.2/81.8[c]
Trimethoprim-sulfamethoxazole		1	>4	48.8/41.5	56.9/41.5		>4	>4	16.7/66.7	27.8/66.7		>4	>4	13.0/87.0	13.0/87.0		>4	>4	13.6/81.8	13.6/81.8
S. pyogenes		*n* = 202					*n* = 203					*n* = 102					*n* = 99			
Nafithromycin		0.015	0.015				0.015	0.015				0.015	0.015				0.015	0.03		
Telithromycin		0.015	0.03		97.0/2.5		0.015	0.03		96.6/3.0		0.015	0.015		99.0/0.0		0.015	0.03		98.0/2.0
Clindamycin		≤0.25	≤0.25	92.6/5.9	94.1/5.9		≤0.25	≤0.25	95.1/4.9	95.1/4.9		≤0.25	≤0.25	97.1/2.9	97.1/2.9		≤0.25	≤0.25	97.0/3.0	97.0/3.0
Erythromycin		≤0.12	8	83.2/16.8	83.2/16.8		≤0.12	0.25	91.1/8.9	91.1/8.9		≤0.12	≤0.12	93.1/5.9	93.1/5.9		≤0.12	≤0.12	94.9/5.1	94.9/5.1
Penicillin		≤0.06	≤0.06	100.0/0.0	100.0/0.0		≤0.06	≤0.06	100.0/0.0	100.0/0.0		≤0.06	≤0.06	100.0/0.0	100.0/0.0		≤0.06	≤0.06	100.0/0.0	100.0/0.0
Erythromycin-resistant and clindamycin-resistant *S. pyogenes*	15					10					3					3				
Nafithromycin		0.015	0.25				0.12	0.5				0.015					0.5			
Telithromycin		0.015	2		66.7/33.3		0.5	>2		40.0/50.0		0.015			66.7/0.0		2			33.3/66.7
Clindamycin		>2	>2	0.0/80.0	20.0/80.0		>2	>2	0.0/100.0	0.0/100.0		>2		0.0/100.0	0.0/100.0		>2		0.0/100.0	0.0/100.0
Erythromycin		>16	>16	0.0/100.0	0.0/100.0		>16	>16	0.0/100.0	0.0/100.0		8		0.0/100.0	0.0/100.0		>16		0.0/100.0	0.0/100.0
Penicillin		≤.06	≤0.06	100.0/0.0	100.0/0.0		≤0.06	≤0.06	100.0/0.0	100.0/0.0		≤0.06		100.0/0.0	100.0/0.0		≤0.06		100.0/0.0	100.0/0.0
H. influenzae	398					402					100					102				
Nafithromycin		4	4				4	4				2	4				2	4		
Telithromycin		2	4	96.7/1.3	0.5/1.3		2	4	98.8/0.5	0.0/0.5		2	4	98.0/1.0	0.0/1.0		2	4	99.0/1.0	99.0/1.0
Amoxicillin-clavulanate		≤1	2	99.7/0.3	99.0/1.0		≤1	≤1	100.0/0.0	99.3/0.7		≤1	≤1	100.0/0.0	99.0/1.0		≤1	4	97.1/2.9	85.3/14.7
Ampicillin		≤0.25	>8	72.4/26.9	72.4/27.6[b]		≤0.25	>8	84.1/13.9	84.1/15.9[b]		≤0.25	>8	78.0/21.0	78.0/22.0[b]		1	>8	65.7/26.5	65.7/34.3[b]
Azithromycin		0.5	2	98.7/	2.3/1.3		0.5	1	100.0/0.0	3.0/0.0		0.5	1	99.0/	4.0/1.0		0.5	1	99.0/	2.0/1.0
Ciprofloxacin		≤0.03	≤0.03	100.0/0.0	99.5/0.5		≤0.03	≤0.03	99.8/	99.3/0.7		≤0.03	≤0.03	100.0/0.0	100.0/0.0		≤0.03	≤0.03	97.1/	96.1/3.9
Clarithromycin		4	8	93.2/1.8	2.8/0.0		4	8	97.0/0.7	2.8/0.0		4	8	99.0/1.0	4.0/0.0		4	8	97.1/2.0	2.0/0.0
Trimethoprim-sulfamethoxazole		≤0.5	>4	65.6/27.4	65.6/32.7		≤0.5	>4	68.4/25.1	68.4/29.6		≤0.5	≥4	71.0/24.0	71.0/28.0		≤0.5	>4	59.8/38.2	59.8/39.2
M. catarrhalis	197					202					54					51				
Nafithromycin		0.12	0.25				0.12	0.25				0.12	0.25				0.25	0.25		
Telithromycin		0.06	0.12		99.0/0.0		0.12	0.12		100.0/0.0		0.06	0.12		100.0/0.0		0.12	0.25		100.0/0.0
Amoxicillin-clavulanate		≤1	≤1	100.0/0.0	100.0/0.0		≤1	≤1	100.0/0.0	100.0/0.0		≤1	≤1	100.0/0.0	100.0/0.0		<1	<1	100.0/0.0	100.0/0.0
Azithromycin		0.03	0.06	100.0/0.0	100.0/0.0		0.03	0.06	100.0/0.0	100.0/0.0		≤0.03	0.06	100.0/0.0	100.0/0.0		0.03	0.06	100.0/0.0	100.0/0.0
Clarithromycin		≤0.12	≤0.12	100.0/0.0	100.0/0.0		≤0.12	≤0.12	100.0/0.0	99.5/0.0		≤0.12	≤0.12	100.0/0.0	100.0/0.0		≤0.12	≤0.12	100.0/0.0	98.0/0.0
Trimethoprim-sulfamethoxazole		≤0.5	≤0.5	94.4/0.0	94.4/3.6		≤0.5	≤0.5	94.6/0.0	94.6/0.5		≤0.5	≤0.5	100.0/0.0	100.0/0.0		≤0.5	≤0.5	98.0/0.0	98.0/0.0

[a]Breakpoint interpretive criteria following the respective guidelines were used.
[b]Using nonmeningitis breakpoints.
[c]Using meningitis breakpoints.
[d]Using oral breakpoints.
[e]Using parenteral, nonmeningitis breakpoints.
[f]MIC, μg/ml.

penicillin V among *S. pneumoniae* isolates was 16.7% overall, with rates of 29.1% in APAC, 11.9% in the EU, 13.6% in the US, and 26.8% in LATAM (Table 2). Nafithromycin was 4-fold less active against penicillin-intermediate and -resistant isolates than against penicillin-susceptible isolates, with nafithromycin $MIC_{50/90}$ values of 0.06/0.12, 0.06/0.12, and 0.015/0.03 μg/ml, respectively (Table 1).

Nafithromycin showed potent activity ($MIC_{50/90}$, 0.015/0.015 μg/ml) against *Streptococcus pyogenes*, inhibiting 100.0% of isolates, with an MIC value of $\leq$0.5 μg/ml (Table 1). Nafithromycin $MIC_{50/90}$ values (0.015/0.015 to 0.03 μg/ml) were similar for the 4 geographic regions (Table 2). Erythromycin resistance was only 10.4% overall (data not shown), with rates ranging from 5.1% in APAC to 16.8% in the US (Table 2). Nafithromycin ($MIC_{50/90}$, 0.015/0.015 μg/ml) demonstrated up to 2-fold greater activity than that of telithromycin ($MIC_{50/90}$, 0.015/0.03 μg/ml) (data not shown).

Against *Haemophilus influenzae*, nafithromycin demonstrated moderate activity ($MIC_{50/90}$, 4/4 μg/ml) (Table 2). $MIC_{50/90}$ results were similar (4/4 μg/ml) for the 4 geographic regions and regardless of β-lactamase production (Table 2). Nafithromycin activity ($MIC_{50/90}$, 4/4 μg/ml) showed equal or 2-fold less potency than that of telithromycin ($MIC_{50/90}$, 2/4 μg/ml) against *H. influenzae* (Table 2 and data not shown).

Nafithromycin exhibited good activity ($MIC_{50/90}$, 0.12/0.25 μg/ml) against *Moraxella catarrhalis* isolates (Table 1). $MIC_{50/90}$ results for nafithromycin were similar (0.12 to 0.25/0.25 μg/ml) for the 4 geographic regions and were not influenced by β-lactamase production (Table 2). Nafithromycin ($MIC_{50/90}$, 0.12/0.25 μg/ml) demonstrated a potency comparable to that of telithromycin ($MIC_{50/90}$, 0.06 to 0.12/0.12 to 0.25 μg/ml) (Table 2).

DISCUSSION

Multidrug-resistant *S. pneumoniae* isolates are a current concern in community-acquired pneumonia in adults (11–13). These multidrug-resistant organisms are included in the serious threat list in the Centers for Disease Control and Prevention (CDC) 2013 report on current antimicrobial threats (14). The potential for the presence of these resistant organisms affects therapy choices (11, 15).

Ketolides are newer macrolides that are active against macrolide-resistant *S. pneumoniae*. The ketolide class derived its name from the ketone functional group at the 3 position, where traditional macrolides have an L-cladinose sugar attached (6–9). The lack of the L-cladinose moiety is believed to reduce the potential for the induction of inducible macrolide resistance. Additionally, ketolides have a bridged 11,12-aryl side chain that allows for an additional interaction with the 23S ribosome in domain II, at A752 (6, 8, 9). The fluoroketolide solithromycin exhibits tight binding to the ribosome, presumably due to the fluorine at the C-2 position of the 14-membered ring, and therefore provides low MICs for *ermB*-expressing *S. pneumoniae* (7). However, in the past, certain ketolides and fluoroketolides have been reported to be associated with an unacceptable safety profile (9, 16). Nafithromycin has unique structural features, with a lactone ketolide nucleus bearing a 2-pyridine-1,3,4-thiadiazole biaryl side chain that is spaced through a four-atom spacer containing a *cis* double bond and a chiral methyl group. The nonflexible spacer possibly aligns the biaryl ring system, resulting in a favorable interaction with dual 23S rRNA targets. Thus, these structural features enabled nafithromycin to demonstrate potent activity against *ermB*-expressing as well as telithromycin-resistant *S. pneumoniae*, without the need to have a fluorine moiety at the C-2 position (5–10). Moreover, it has been reported that compared to solithromycin, nafithromycin retains a higher degree of activity under hyper-*ermB*-induction conditions in *S. pneumoniae*, indicating a better target interaction even for methylated 23S rRNA (17).

Nafithromycin is a novel antimicrobial agent of the lactone ketolide class that is in clinical development for treatment of CABP. In this report, nafithromycin was shown to be as active *in vitro* as the comparator ketolide, telithromycin (88.4% of isolates were susceptible), against a global collection of *S. aureus* isolates cultured during 2014. The activities of nafithromycin and telithromycin were considerably lowered against *S.*

aureus strains demonstrating a constitutively expressed macrolide resistance phenotype. Nafithromycin was also very active against *S. pneumoniae*, with 100.0% of isolates inhibited, with MIC values of ≤0.25 μg/ml. Nafithromycin also showed excellent activity against *S. pyogenes* (all MIC values were ≤0.5 μg/ml), moderate activity ($MIC_{50/90}$, 4/4 μg/ml) against *H. influenzae*, and potent activity ($MIC_{50/90}$, 0.12/0.25 μg/ml) against *M. catarrhalis*.

In a phase 1 intrapulmonary pharmacokinetic study involving an 800-mg once-daily dose administered over 3 days, nafithromycin showed high lung epithelial lining fluid and alveolar macrophage concentrations that lasted at clinically relevant levels (1.62 ± 0.86 and 22.4 ± 10.4 μg/ml for epithelial lining fluid and alveolar macrophages, respectively) for at least 48 h after the last dose (18). The spectrum of activity of nafithromycin and its chemical characteristics, which allow for the development of oral and intravenous formulations, indicate that further clinical development to treat infections caused by multidrug-resistant Gram-positive organisms is warranted.

MATERIALS AND METHODS

Organisms. A total of 4,739 nonduplicate isolates were selected from a 2014 global surveillance program at medical institutions located in 43 countries within the US, EU, LATAM, and APAC (Table 1). Only clinically significant isolates were included in the study (1 per infection episode). Species identification was performed at the site and confirmed at the central monitoring laboratory, when necessary, by standard biochemical tests and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), using a Bruker Daltonics MALDI Biotyper machine (Billerica, MA, USA) per the manufacturer's instructions. Organisms chosen for this study were a selected group of species generally recognized as respiratory pathogens and were primarily Gram positive.

Antimicrobial susceptibility testing. MIC values were determined using CLSI broth microdilution methods as described in CLSI document M07-A10 (2015) (19). Cation-adjusted Mueller-Hinton broth (CA-MHB) was used for inoculation of MIC panels for all nonfastidious organisms. *Haemophilus influenzae* isolates were tested in *Haemophilus* test medium (HTM). In addition, lysed horse blood (3.75%) was used to supplement the CA-MHB for testing of *S. pneumoniae* and *S. pyogenes*. For nafithromycin and telithromycin, MIC results were obtained using validated broth microdilution panels produced by JMI Laboratories (North Liberty, IA). For other antimicrobial agents, dry-form MIC panels were manufactured by Thermo Fisher Scientific (formerly TREK Diagnostics Systems/Sensititre; Cleveland, OH, USA). MIC value validation was performed by concurrent testing of the following quality control (QC) reference strains: *S. pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *H. influenzae* ATCC 49247. All nafithromycin and telithromycin MIC values were within the QC ranges recommended by the CLSI. MIC interpretations were based on CLSI and EUCAST breakpoint criteria (20, 21).

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