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In Vitro Activity of CEM-101 against *Streptococcus pneumoniae* and *Streptococcus pyogenes* with Defined Macrolide Resistance Mechanisms[▽]

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CEM-101 had MIC ranges of 0.002 to 0.016 μg/ml against macrolide-susceptible pneumococci and 0.004 to 1 μg/ml against macrolide-resistant phenotypes. Only 3 strains with *erm*(B), with or without *mef*(A), had CEM-101 MICs of 1 μg/ml, and 218/221 strains had CEM-101 MICs of ≤0.5 μg/ml. CEM-101 MICs were as much as 4-fold lower than telithromycin MICs against all strains. For *Streptococcus pyogenes*, CEM-101 MICs ranged from 0.008 to 0.03 μg/ml against macrolide-susceptible strains and from 0.015 to 1 μg/ml against macrolide-resistant strains. Against *erm*(B) strains, erythromycin, azithromycin, and clarithromycin MICs were 32 to >64 μg/ml, while 17/19 strains had telithromycin MICs of 4 to 16 μg/ml; CEM-101 MICs were 0.015 to 1 μg/ml. By comparison, *erm*(A) and *mef*(A) strains had CEM-101 MICs of 0.015 to 0.5 μg/ml, clindamycin and telithromycin MICs of ≤1 μg/ml, and erythromycin, azithromycin, and clarithromycin MICs of 0.5 to >64 μg/ml. Pneumococcal multistep resistance studies showed that although CEM-101 yielded clones with higher MICs for all eight strains tested, seven of eight strains had clones with CEM-101 MICs that rose from 0.004 to 0.03 μg/ml (parental strains) to 0.06 to 0.5 μg/ml (resistant clones); for only one *erm*(B) *mef*(A) strain with a parental MIC of 1 μg/ml was there a resistant clone with a MIC of 32 μg/ml, with no detectable mutations in the L4, L22, or 23S rRNA sequence. Among two of five *S. pyogenes* strains tested, CEM-101 MICs rose from 0.03 to 0.25 μg/ml, and only for the one strain with *erm*(B) did CEM-101 MICs rise from 1 to 8 μg/ml, with no changes occurring in any macrolide resistance determinant. CEM-101 had low MICs as well as low potential for the selection of resistant mutants, independent of bacterial species or resistance phenotypes in pneumococci and *S. pyogenes*.

Strains of *Streptococcus pneumoniae* resistant to macrolides, β-lactams, quinolones, and other agents are seen worldwide. Macrolide resistance is now predominant in some countries, such as Japan and Korea, most likely due to overuse of azithromycin and clarithromycin during the past 15 years. Macrolide resistance usually also occurs (although genetically unlinked) together with penicillin G resistance (8, 9, 22). Although all strains of group A streptococci remain β-lactam susceptible, macrolide resistance occurs, especially in Southern, Central, and Eastern Europe and Asia (15, 22, 23).

Although the pediatric conjugate vaccine has dramatically decreased the incidence of meningitis and bacteremia caused by most of the usual drug-resistant pneumococcal clones, recent papers have described the spread of multidrug-resistant pneumococcal strains with a serotype (19A), not included in the vaccine, which causes otitis media that is not amenable to treatment with any currently available Food and Drug Administration-approved antibiotic (9, 19, 24). The problem of drug-resistant pneumococci causing community-acquired respiratory infection, especially in children, is likely to worsen with the spread of this clone.

The introduction of telithromycin into the therapeutic armamentarium was, with the exception of *erm*(B) group A streptococci (which are naturally telithromycin resistant), intended to solve the problem of macrolide resistance in streptococci (2, 21, 22). However, safety issues have limited the clinical utility of this drug. Additionally, when the free area under the curve (AUC)/MIC ratio of telithromycin against macrolide-resistant pneumococci was examined carefully, even with low MICs, it could be seen that the number was not significantly above 25; thus, resistance was predicted, and this has indeed been the case, as evidenced by recent publications (25).

CEM-101 (Fig. 1) is a novel fluoroketolide containing an 11,12-carbamate-butyl-[1,2,3]-triazolyl-aminophenyl side chain. CEM-

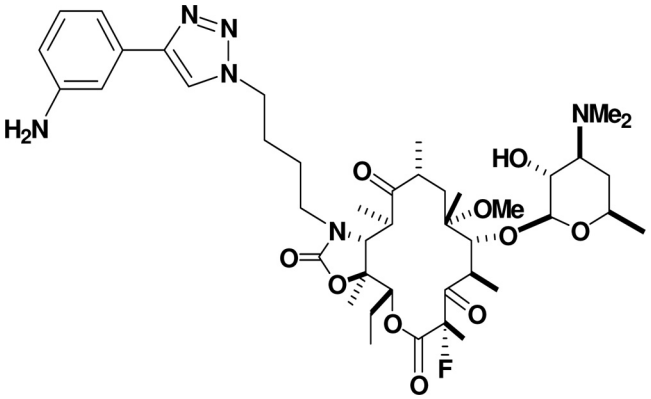

FIG. 1. Structure of CEM-101.

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TABLE 1. MICs of drugs against 221 pneumococcal strains

Drug and type of strain tested[a]	MIC (μg/ml)[b]			Drug and type of strain tested[a]	MIC (μg/ml)[b]		
	Range	50%	90%		Range	50%	90%
Penicillin G	0.008–>16	1	4	Telithromycin	0.015–2	0.06	0.5
Penicillin S	0.008–0.06	0.03	0.06	Penicillin S	0.015–1	0.06	0.25
Penicillin I	0.125–1	0.5	1	Penicillin I	0.015–1	0.06	0.5
Penicillin R	2–>16	4	8	Penicillin R	0.015–2	0.125	0.5
Macrolide S	0.015–8	1	2	Macrolide S	0.015–0.03	0.03	0.03
erm(B)	0.03–16	1	4	*erm*(B)	0.03–2	0.06	1
mef(A)	0.008–4	0.125	4	*mef*(A)	0.03–0.5	0.125	0.25
erm(A)	0.03–0.03			*erm*(A)	0.03–0.06		
erm(B) *mef*(A)	0.03–8	2	4	*erm*(B) *mef*(A)	0.03–2	0.5	1
L4 mutations	1–>16	4	16	L4 mutations	0.06–0.25	0.125	0.25
23S rRNA mutations	0.015–0.5			23S rRNA mutations	0.03–0.06		
Quinolone S	0.008–>16	1	4	Quinolone S	0.015–2	0.125	0.5
Quinolone R	0.015–8	0.25	4	Quinolone R	0.015–1	0.03	0.125
CEM-101	0.002–1	0.03	0.25	Clindamycin	0.015–>64	0.06	>64
Penicillin S	0.002–0.25	0.03	0.125	Penicillin S	0.03–>64	0.06	>64
Penicillin I	0.002–0.25	0.03	0.25	Penicillin I	0.03–>64	0.125	>64
Penicillin R	0.004–1	0.06	0.25	Penicillin R	0.015–>64	0.06	>64
Macrolide S	0.002–0.015	0.008	0.015	Macrolide S	0.015–0.06	0.03	0.06
erm(B)	0.004–1	0.03	0.5	*erm*(B)	0.06–>64	>64	>64
mef(A)	0.008–0.25	0.03	0.125	*mef*(A)	0.03–0.125	0.06	0.06
erm(A)	0.008–0.015	-----	-----	*erm*(A)	0.125–0.25		
erm(B) *mef*(A)	0.015–1	0.125	0.25	*erm*(B) *mef*(A)	0.03–>64	0.06	>64
L4 mutations	0.03–0.125	0.06	0.125	L4 mutations	0.03–0.125	0.06	0.125
23S rRNA mutations	0.002–0.03			23S rRNA mutations	0.03–1		
Quinolone S	0.002–1	0.03	0.25	Quinolone S	0.015–>64	0.06	>64
Quinolone R	0.004–0.25	0.008	0.06	Quinolone R	0.03–64	0.03	64
Erythromycin	0.03–>64	64	>64	Amoxicillin-clavulanate	0.015–16	0.05	8
Penicillin S	0.03–>64	4	>64	Penicillin S	0.015–0.125	0.03	0.06
Penicillin I	0.03–>64	>64	>64	Penicillin I	0.03–2	0.5	1
Penicillin R	0.03–>64	>64	>64	Penicillin R	0.125–16	2	8
Macrolide S	0.03–0.25	0.06	0.125	Macrolide S	0.015–8	0.5	2
erm(B)	16–>64	>64	>64	*erm*(B)	0.015–8	0.5	8
mef(A)	1–>64	4	32	*mef*(A)	0.015–8	0.125	2
erm(A)	2–4			*erm*(A)	0.03–0.03		
erm(B) *mef*(A)	4–>64	>64	>64	*erm*(B) *mef*(A)	0.03–16	2	8
L4 mutations	4–>64	>64	>64	L4 mutations	0.125–8	4	8
23S rRNA mutations	8–>64			23S rRNA mutations	0.03–0.06		
Quinolone S	0.03–>64	>64	>64	Quinolone S	0.015–16	1	8
Quinolone R	0.03–>64	0.06	>64	Quinolone R	0.015–4	0.5	2
Azithromycin	0.06–>64	16	>64	Levofloxacin	0.06–32	1	8
Penicillin S	0.06–>64	4	>64	Penicillin S	0.06–32	1	16
Penicillin I	0.06–>64	>64	>64	Penicillin I	1–32	1	2
Penicillin R	0.06–>64	>64	>64	Penicillin R	0.5–16	1	2
Macrolide S	0.06–0.25	0.125	0.0125	Macrolide S	1–32	1	16
erm(B)	>64–>64	>64	>64	*erm*(B)	0.5–32	1	2
mef(A)	1–>64	4	8	*mef*(A)	0.5–8	1	2
erm(A)	2–8			*erm*(A)	1–1		
erm(B) *mef*(A)	2–>64	>64	>64	*erm*(B) *mef*(A)	1–16	1	16
L4 mutations	2–>64	>64	>64	L4 mutations	0.5–16	1	2
23S rRNA mutations	32–>64			23S rRNA mutations	0.06–1		
Quinolone S	0.06–>64	>64	>64	Quinolone S	0.06–2	1	2
Quinolone R	0.06–>64	0.125	>64	Quinolone R	4–32	16	16
Clarithromycin	0.125–>64	8	>64	Moxifloxacin	0.125–8	0.25	2
Penicillin S	0.015–>64	1	>64	Penicillin S	0.125–8	0.5	4
Penicillin I	0.03–>64	16	>64	Penicillin I	0.125–4	0.25	0.5
Penicillin R	0.015–>64	16	>64	Penicillin R	0.125–4	0.25	0.5
Macrolide S	0.015–0.06	0.03	0.06	Macrolide S	0.125–8	0.25	4
erm(B)	4–>64	>64	>64	*erm*(B)	0.125	0.25	0.5
mef(A)	0.5–32	2	4	*mef*(A)	0.125–4	0.25	0.5
erm(A)	0.25–0.5			*erm*(A)	0.25–0.5		
erm(B) *mef*(A)	1–>64	>64	>64	*erm*(B) *mef*(A)	0.125–2	0.25	0.5
L4 mutations	1–32	16	32	L4 mutations	0.125–4	0.25	0.5
23S rRNA mutations	8–16			23S rRNA mutations	0.25–0.5		
Quinolone S	0.015–>64	16	>64	Quinolone S	0.125–1	0.25	0.5
Quinolone R	0.015–>64	0.03	>64	Quinolone R	0.5–8	4	4

[a] S, susceptible; I, intermediate; R, resistant. A total of 53 penicillin-susceptible, 63 penicillin-intermediate, 105 penicillin-resistant, 50 macrolide-susceptible, 54 *erm*(B), 51 *mef*(A), 4 *erm*(A), and 31 *erm*(B) *mef*(A) strains, 27 strains with L4 mutations, 4 strains with 23S rRNA mutations, 195 quinolone-susceptible strains, and 27 quinolone-resistant strains were tested.

[b] 50% and 90%, MICs at which 50% and 90% of isolates, respectively, are inhibited.

TABLE 2. MIC_{50}s and MIC_{90}s[a] of pneumococcal strains with defined macrolide-resistant mechanisms

Drug	MIC (μg/ml) for strains with the following macrolide-resistant mechanism (no. of strains):							
	erm(B) (54)		*mef*(A) (51)		*erm*(B) *mef*(A) (31)		L4 mutations (27)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
CEM-101	0.03	0.5	0.03	0.125	0.125	0.25	0.06	0.125
Erythromycin	>64	>64	4	32	>64	>64	>64	>64
Azithromycin	>64	>64	4	8	>64	>64	>64	>64
Clarithromycin	>64	>64	2	4	>64	>64	16	32
Telithromycin	0.06	1	0.125	0.25	0.5	1	0.125	0.25
Clindamycin	>64	>64	0.06	0.06	0.06	>64	0.06	0.125
Amoxicillin-clavulanate	0.5	8	0.125	2	2	8	4	8
Levofloxacin	1	2	1	2	1	16	1	2
Moxifloxacin	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5
Penicillin G	1	4	0.125	4	2	4	4	16

[a] MIC_{50} and MIC_{90}, MICs at which 50% and 90% of isolates, respectively, are inhibited.

101 demonstrates enhanced potency compared to telithromycin, with activity against telithromycin-intermediate and telithromycin-resistant organisms (1, 7, 10–14, 17,18, 20, 26–29). In the current study, we have performed (i) MIC studies to compare the activity of CEM-101 to those of erythromycin, azithromycin, clarithromycin, telithromycin, clindamycin, penicillin G, amoxicillin-clavulanate, levofloxacin, and moxifloxacin against a spectrum of pneumococci and group A streptococci with different macrolide resistance phenotypes and genotypes, and (ii) single and multistep resistance studies to examine the ability of CEM-101 to select for resistant mutants of pneumococci and group A streptococci compared to those of telithromycin, azithromycin, clarithromycin, and clindamycin.

TABLE 3. MICs of drugs against 124 group A streptococci

Drug and type of strain[a]	MIC (μg/ml)[b]			Drug and type of strain[a]	MIC (μg/ml)[b]		
	Range (μg/ml)	50%	90%		Range (μg/ml)	50%	90%
CEM-101	0.008–1	0.06	0.5	Amoxicillin-clavulanate	<0.015–0.125	0.03	0.03
Macrolide S	0.008–0.03	0.015	0.03	Macrolide S	0.015–0.03	0.03	0.03
erm(B)	0.03–1	0.5	1	*erm*(B)	<0.015–0.125	<0.015	0.03
mef(A)	0.06–0.25	0.125	0.25	*mef*(A)	<0.015–0.125	0.015	0.06
erm(A)	0.016–0.5	0.03	0.125	*erm*(A)	<0.015–0.03	0.03	0.03
L4 mutations	0.06			L4 mutations	0.03		
Erythromycin	0.03–>64	16	>64	Levofloxacin	0.5–2	0.5	1
Macrolide S	0.03–0.25	0.06	0.125	Macrolide S	0.5–1	0.5	0.5
erm(B)	>64–>64	>64	>64	*erm*(B)	0.5–1	0.5	1
mef(A)	8–32	16	32	*mef*(A)	0.5–2	0.5	1
erm(A)	2–>64	4	>64	*erm*(A)	0.5–2	0.5	1
L4 mutations	2			L4 mutations	0.5		
Azithromycin	0.06–>64	8	>64	Moxifloxacin	0.0125–0.5	0.25	0.25
Macrolide S	0.06–0.25	0.125	0.25	Macrolide S	0.125–0.25	0.25	0.25
erm(B)	>64–>64	>64	>64	*erm*(B)	0.125–0.25	0.25	0.25
mef(A)	0.5–16	8	8	*mef*(A)	0.25–0.5	0.25	0.25
erm(A)	2–>64	16	>64	*erm*(A)	0.125–0.5	0.25	0.25
L4 mutations	2			L4 mutations	0.25		
Clarithromycin	0.015–>64	4	>64	Penicillin G	<0.008–0.125	0.015	0.015
Macrolide S	0.015–0.06	0.03	0.06	Macrolide S	0.008–0.015	0.015	0.015
erm(B)	32–>64	>64	>64	*erm*(B)	<0.008–0.125	0.015	0.015
mef(A)	0.5–8	4	8	*mef*(A)	<0.008–0.125	0.015	0.03
erm(A)	0.25–>64	2	>64	*erm*(A)	<0.008–0.015	0.015	0.015
L4 mutations	1			L4 mutations	0.015		
Telithromycin	0.03–16	0.125	8	Clindamycin	0.03–>64	0.125	>64
Macrolide S	0.03–0.06	0.06	0.06	Macrolide S	0.03–0.125	0.06	0.06
erm(B)	0.03–16	8	16	*erm*(B)	0.06–>64	>64	>64
mef(A)	0.125–1	0.5	1	*mef*(A)	0.03–0.125	0.06	0.125
erm(A)	0.03–0.25	0.06	0.125	*erm*(A)	0.06–0.5	0.125	0.25
L4 mutations	0.06			L4 mutations	0.06		

[a] S, susceptible. A total of 26 macrolide-susceptible, 19 *erm*(B), 38 *mef*(A), and 40 *erm*(A) strains, as well as 1 strain with L4 mutations, were tested.
[b] 50 and 90%, MICs at which 50% and 90% of isolates, respectively, are inhibited.

TABLE 4. MIC_{50}s and MIC_{90}s[a] of group A streptococcal strains with defined macrolide-resistant mechanisms

Drug	MIC (μg/ml) for strains with the following macrolide-resistant mechanism (no. of strains):					
	erm(B) (19)		*mef*(A) (38)		*erm*(A) (40)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
CEM-101	0.5	1	0.125	0.25	0.03	0.125
Erythromycin	>64	>64	16	32	4	>64
Azithromycin	>64	>64	8	8	16	>64
Clarithromycin	>64	>64	4	8	2	>64
Telithromycin	8	16	0.5	1	0.06	0.125
Clindamycin	>64	>64	0.06	0.125	0.125	0.25
Amoxicillin-clavulanate	<0.015	0.03	0.015	0.06	0.03	0.03
Levofloxacin	0.5	1	0.5	1	0.5	1
Moxifloxacin	0.25	0.25	0.25	0.25	0.25	0.25
Penicillin G	0.015	0.015	0.015	0.03	0.015	0.015

[a] MIC_{50} and MIC_{90}, MICs at which 50% and 90% of isolates, respectively, are inhibited.

MATERIALS AND METHODS

Bacteria and antimicrobials. We tested 221 clinical pneumococcal strains by MIC testing. These comprised 50 macrolide-susceptible and 171 macrolide-resistant organisms. Macrolide-resistant strains all had defined genotypes and comprised strains with *erm*(B) (54 strains), *mef*(A) (51 strains), *erm*(B) plus *mef*(A) (31 strains), *erm*(A) (4 strains), and mutations in the L4 ribosomal protein (27 strains) and 23S rRNA (4 strains). These 221 strains also comprised 27 non-quinolone-susceptible phenotypes with defined quinolone resistance determinant regions (QRDRs) (levofloxacin MICs, 4 to 32 μg/ml) and the entire spectrum of penicillin G resistance phenotypes according to the latest Clinical and Laboratory Standards Institute (CLSI) oral penicillin V susceptibility classification (4). The 124 group A streptococci for which MICs were determined comprised 26 macrolide-susceptible and 98 macrolide-resistant strains. The latter comprised 19 strains with *erm*(B), 38 with *mef*(A), 40 with *erm*(A), and 1 with an L4 mutation. Because strains of both species studied were chosen for their macrolide resistance phenotypes, only susceptible strains were consistently recent (2003 to 2008) isolates; some resistant strains were isolated up to 5 years earlier (1998). *Streptococcus pneumoniae* ATCC 49619 was included as the quality control strain for each species and each run (5).

For resistance selection testing, one each of the following pneumococcal resistance phenotypes was tested: macrolide susceptible, *erm*(B) positive, *mef*(A) positive, *erm*(B) and *mef*(A) positive, *erm*(A) positive, and with mutations in ribosomal proteins (L4, L22) and 23S rRNA. Five strains of group A streptococci were tested; one each was macrolide susceptible, *erm*(B) positive, *mef*(A) positive, *erm*(A) positive, or had an L4 mutation. CEM-101 was obtained from Cempra Pharmaceuticals, Chapel Hill, NC, and other drugs were obtained either from their respective manufacturers or from Sigma Chemical, Inc., St. Louis, MO.

MIC determinations. MICs were determined by the agar dilution technique, which, though not specifically recommended by the CLSI (5), has been in use in our research laboratory for >20 years (3, 6, 16). Mueller-Hinton agar (BD Diagnostics, Sparks, MD) supplemented with 5% sheep blood agar was used, with 10^4 CFU/spot and overnight incubation at 35°C in ambient air. The usual quality control strains were included in each run (5). For resistance selection, CLSI macrodilution (5) was used for MIC testing.

Mechanism of macrolide resistance. All macrolide-resistant parental strains were tested for the presence of the *erm*(B), *erm*(A), *mef*(E), and *mef*(A) genes by PCR amplification (3, 5, 21, 22). All parental isolates and CEM-101-resistant clones (CEM-101 MIC, >1 μg/ml) were examined for the presence of mutations in the L4 and L22 ribosomal proteins and 23S rRNA (II and V domains) by using the primers and conditions described previously (2, 3, 6, 16, 21, 22). The nucleotide sequences were obtained by direct sequencing with a CEQ8000 genetic analysis system (Beckman Coulter, Fullerton, CA).

Multistep resistance selection. Serial passages of each strain were performed daily in subinhibitory concentrations of all antimicrobials. In all cases, the broth medium was 1 ml per tube of cation-adjusted Mueller-Hinton broth (BD Diagnostics, Sparks, MD) plus 5% lysed horse blood. For each subsequent daily passage, an inoculum (10 μl) was taken from the tube at 1 to 2 dilutions below the MIC that matched the turbidity of a growth control tube. This inoculum was used to determine the next MIC. Daily passages were performed until a significant increase in the MIC (≥8 times) was obtained. A minimum of 14 passages were performed unless MICs of ≥32 μg/ml were obtained. The maximal number of passages was 50. The stability of the acquired resistance was ascertained by MIC determinations after 10 daily passages of the mutants on blood agar without antibiotics. The MIC of each compound for each resistant pneumococcal clone was determined by macrodilution. The identities of the mutants obtained and their respective parents were confirmed by pulsed-field gel electrophoresis (PFGE) at the end of the study. PFGE of SmaI-digested DNA was performed using a CHEF DR III apparatus (Bio-Rad, Hercules, CA) with the following run parameters: a switch time of 5 to 20 s and a run time of 16 h (3, 6, 16).

Single-step studies. The frequency of spontaneous single-step mutations was determined by spreading suspensions (approximately 10^{10} CFU/ml) on Mueller-Hinton agar (BD Diagnostics, Sparks, MD) with 5% sheep blood at 2, 4, and 8 times the MIC (2×, 4×, and 8× MIC). After incubation at 35°C under 5% CO_2 for 48 h, the frequency of resistance was calculated as the number of colonies per inoculum for which the MIC was at least 4 times higher than the MIC for the parental strain. Single-step studies were not performed with azithromycin, clarithromycin, clindamycin, and telithromycin for strains with MICs of ≥4 μg/ml (3, 6, 16).

RESULTS

The results of pneumococcal MIC testing are presented in Tables 1 and 2. As can be seen, CEM-101 MICs ranged from 0.002 to 0.015 μg/ml against macrolide-susceptible pneumococci and from 0.004 to 1 μg/ml against macrolide-resistant pneumococci (all phenotypes). Only 3 strains with *erm*(B) [with or without *mef*(A)] had CEM-101 MICs of 1.0 μg/ml, and 218/221 strains had CEM-101 MICs of ≤0.5 μg/ml. In contrast, corresponding telithromycin MICs ranged from 0.015 to 0.03 μg/ml for macrolide-susceptible strains and from 0.015 to 2 μg/ml for macrolide-resistant strains. CEM-101 MICs were as much as fourfold lower than telithromycin MICs against macrolide-susceptible and -resistant strains.

All group A streptococcal strains were penicillin G susceptible. MICs are presented in Tables 3 and 4. CEM-101 MICs were 0.008 to 0.03 μg/ml against macrolide-susceptible strains and 0.015 to 1 μg/ml against macrolide-resistant strains (all phenotypes). Telithromycin MICs were as much as fourfold higher than CEM-101 MICs. Importantly, 17/19 *erm*(B) strains were telithromycin resistant, with MICs between 4 and 16 μg/ml, while all had low CEM-101 MICs, similar to those of strains with other resistance phenotypes (range, 0.03 to 1 μg/ml).

The results of pneumococcal multistep resistance selection studies are presented in Table 5. As can be seen for pneumococci, parental MICs (in micrograms per milliliter) were as follows: CEM-101, 0.004 to 1; azithromycin, 0.03 to 8; clar-

TABLE 5. *S. pneumoniae* multistep selection results[a]

Strain no.	Phenotype (resistance determinant)[a]	Drug[b]	Initial MIC (μg/ml)	Selected resistance		Retest MIC[c] after passages in subinhibitory concns of the following antibiotic and 10 antibiotic-free subcultures:				
				MIC (μg/ml)	No. of passages	CEM	AZI	CLA	TEL	CLI
1077	Macrolide S	CEM	0.008	0.06	43	0.06	0.03	0.06	**0.06**	0.03
		AZI	0.03	>64	29	0.03	>64	**>64**	**0.125**	**4**
		CLA	0.016	0.008	50					
		TEL	0.004	0.25	15	**0.06**	**>64**	**>64**	0.25	**8**
		CLI	0.016	4	49	**0.06**	**>64**	**>64**	**0.06**	8
24	Macrolide R [*erm*(B)]	CEM	0.004	0.06	14	0.06	>64	>64	0.125	>64
		AZI	>64	NT[d]	NT					
		CLA	>64	NT	NT					
		TEL	0.25	32	14	**0.06**	>64	>64	16	>64
		CLI	>64	NT	NT					
3665	Macrolide R [*mef*(A)]	CEM	0.03	0.5	14	0.5	16	4	0.25	0.03
		AZI	8	16	50					
		CLA	2	16	26	0.06	8	8	0.06	0.03
		TEL	0.125	2	14	0.03	4	2	0.25	0.03
		CLI	0.016	0.03	50					
1076	Macrolide-R [*erm*(B) *mef*(A)]	CEM	1	32	18	32	>64	>64	**32**	>64
		AZI	>64	NT	NT					
		CLA	>64	NT	NT					
		TEL	0.5	>64	14	2	>64	>64	>64	>64
		CLI	64	NT	NT					
1635	Macrolide R [*erm*(A)]	CEM	0.008	0.06	32	0.125	4	1	**0.03**	0.03
		AZI	2	>64	14	0.004	>64	**>64**	0.004	0.03
		CLA	0.5	>64	49	0.008	**>64**	>64	0.016	0.25
		TEL	0.004	0.008	50					
		CLI	0.06	>64	14	0.004	4	0.5	0.008	>64
2686	Macrolide R (L4 mutation)	CEM	0.03	0.5	22	1	>64	32	0.25	0.03
		AZI	>64	NT	NT					
		CLA	8	>64	14	0.03	>64	>64	0.06	0.03
		TEL	0.06	0.5	25	0.016	>64	16	0.5	0.03
		CLI	0.03	0.125	50					
7127	Macrolide S (S20N in L4, A105V in L22)	CEM	0.008	0.125	16	0.06	0.06	0.125	**0.06**	0.03
		AZI	0.06	0.5	29	0.016	1	**0.5**	0.016	0.06
		CLA	0.03	16	15	0.008	**>64**	16	0.008	**1**
		TEL	0.008	0.06	38	0.008	0.06	0.06	0.03	0.03
		CLI	0.03	0.25	43	0.004	0.03	0.016	0.008	0.25
3009	Macrolide R (23S rRNA mutation)	CEM	0.016	0.25	20	0.25	>64	**>64**	0.06	1
		AZI	>64	NT	NT					
		CLA	16	>64	25	0.03	>64	64	0.06	1
		TEL	0.016	0.03	50					
		CLI	1	2	50					

[a] S, susceptible; R, resistant.
[b] CEM, CEM-101; AZI, azithromycin; CLA, clarithromycin; TEL, telithromycin; CLI, clindamycin.
[c] Boldface indicates cross-reactivity.
[d] NT, not tested.

ithromycin, 0.016 to 16; telithromycin, 0.004 to 0.5; clindamycin, 0.016 to 1. Four, two, and two strains with azithromycin, clarithromycin, and clindamycin MICs of ≥64 μg/ml, respectively, were not tested. CEM-101 MICs increased after 14 to 43 days for all eight strains tested. For seven strains, MICs rose from 0.004 to 0.03 μg/ml (parents) to 0.06 to 0.5 μg/ml (resistant clones) in 14 to 43 days. For the eighth strain, containing *erm*(B) plus *mef*(A), MICs rose from 1 μg/ml (parent) to 32 μg/ml (resistant clone) in 18 days. This CEM-101-resistant clone was subjected to sequencing analysis, which revealed no alterations from parental sequences in the L4 and L22 proteins and in domains II and V of 23S rRNA. Azithromycin produced resistant clones after 14 to 29 days for three of four strains, with MICs rising from 0.03 to 2 μg/ml (parents) to 0.5 to >64 μg/ml (resistant clones). Clarithromycin produced resistant clones after 14 to 49 days for five of six strains, with MICs rising from 0.03 to 16 μg/ml (parents) to 16 to >64 μg/ml (resistant clones). Telithromycin produced stable resistant clones after

TABLE 6. *S. pyogenes* multistep selection results

Strain no.	Phenotype (resistance determinant)[a]	Antibiotic[b]	Initial MIC (μg/ml)	Selected resistance		Retest MIC (μg/ml)[c] after passages in subinhibitory concns of the following antibiotic and 10 antibiotic-free subcultures:				
				MIC (μg/ml)	No. of passages	CEM	AZI	CLA	TEL	CLI
2132	Macrolide S	CEM	0.008	0.016	50					
		AZI	0.06	1	28	0.016	1	**0.25**	0.03	0.03
		CLA	0.03	0.016	50					
		TEL	0.008	0.03	50					
		CLI	0.06	0.06	50					
2368	Macrolide R [*erm*(B)]	CEM	1	8	18	8	>64	>64	**>64**	>64
		AZI	>64	NT[d]	NT					
		CLA	>64	NT	NT					
		TEL	8	>64	6	0.5	>64	>64	>64	>64
		CLI	>64	NT	NT					
2094	Macrolide R [*erm*(A)]	CEM	0.03	0.25	43	0.25	4	**8**	**0.5**	0.06
		AZI	4	>64	5	0.016	>64	1	0.03	0.06
		CLA	0.5	>64	6	0.016	**>64**	>64	0.03	0.06
		TEL	0.03	0.25	22	0.03	**>64**	**8**	0.125	**>64**
		CLI	0.06	>64	34	0.03	16	1	0.03	>64
2011	Macrolide R [*mef*(A)]	CEM	0.125	0.125	50					
		AZI	4	32	35	0.06	16	4	0.25	0.06
		CLA	4	8	50					
		TEL	0.5	1	50					
		CLI	0.06	0.06	50					
237	Macrolide R (L4 mutation)	CEM	0.03	0.25	20	0.5	4	1	**1**	0.03
		AZI	4	8	50					
		CLA	0.25	1	50					
		TEL	0.06	0.125	50					
		CLI	0.06	0.5	43	0.03	8	0.5	0.06	1

[a] S, susceptible; R, resistant.
[b] CEM, CEM-101; AZI, azithromycin; CLA, clarithromycin; TEL, telithromycin; CLI, clindamycin.
[c] Boldface indicates cross-reactivity.
[d] NT, not tested.

14 to 38 days for five of eight strains tested, with MICs rising from 0.004 to 0.5 μg/ml (parents) to 0.06 to >64 μg/ml (resistant clones). Clindamycin produced resistant clones after 14 to 43 days for two of five strains, with MICs rising from 0.03 to 0.06 μg/ml (parents) to 0.25 to >64 μg/ml (resistant clones).

For *Streptococcus pyogenes* (Table 6), parental MICs (μg/ml) were: CEM-101, 0.008 to 1; azithromycin, 0.06 to 4; clarithromycin, 0.03 to 4; telithromycin, 0.008 to 8; clindamycin 0.06. One strain with azithromycin, clarithromycin and clindamycin MICs >64 μg/ml was not tested. CEM-101 MICs increased after 18 to 43 days in 3/5 strains, rising from 0.03 to 1 μg/ml (parents) to 0.25 to 8 μg/ml (resistant clones). The resistant clone with a CEM-101 MIC of 8 μg/ml was subjected to sequencing analysis, which showed no changes in all genes (L4, L22 and II and V domain of 23S rRNA) tested. CEM-101 MICs for the remaining 2 clones did not go above 0.25 μg/ml when passages were continued for the maximum 50 days. Azithromycin had resistant clones after 5 to 35 days in 3/4 strains tested, with MICs rising from 0.06 to 4 μg/ml (parents) to 1 to >64 μg/ml (resistant clones). Clarithromycin had resistant clones after 6 days in 1/4 strains tested, with MICs rising from 0.5 μg/ml (parent) to >64 μg/ml (resistant clone). Telithromycin had resistant clones after 6 to 22 days in 2/5 strains tested, with MICs rising from 0.03 to 8 μg/ml (parents) to 0.25 to >64 μg/ml (resistant clones). Clindamycin had resistant clones after 34 to 43 days in 2/4 strains tested with MICs rising from 0.06 μg/ml (parents) to 0.5 to >64 μg/ml (resistant clones).

The results of single-step resistance selection studies for pneumococci are presented in Table 7. The same four comparators used in multistep selection were tested for their propensities to produce spontaneous mutations. Mutant selection frequencies for CEM-101 ranged from $<2.0 \times 10^{-10}$ to 6.8×10^{-7} at 2× MIC to $<2.0 \times 10^{-10}$ to 9.1×10^{-9} at 8× MIC. These comparators of CEM-101 had higher frequencies of resistance: telithromycin, 1.1×10^{-9} to 1.3×10^{-4} at 2× MIC to $<1.5 \times 10^{-10}$ to 4.8×10^{-6} at 8× MIC; clindamycin, $<2.4 \times 10^{-10}$ to 1.7×10^{-4} at 2× MIC to $<1.2 \times 10^{-10}$ to 5.6×10^{-7} at 8× MIC; and clarithromycin, $<1.0 \times 10^{-9}$ to 5.0×10^{-7} at 2× MIC to $<1.2 \times 10^{-10}$ to $<3.1 \times 10^{-9}$ at 8× MIC. A small number, three strains, were tested with azithromycin; mutant selection frequencies were $<2.0 \times 10^{-10}$ to 7.2×10^{-9} at 2× MIC to $<1.9 \times 10^{-10}$ to $<2.0 \times 10^{-10}$ at 8× MIC.

The results of single-step resistance selection studies for *S. pyogenes* are presented in Table 8. As with the pneumococci, the four comparators used in multistep selection were tested for their propensities to produce spontaneous mutations. Mu-

TABLE 7. *S. pneumoniae* single-step mutation frequencies

Strain no.	Phenotype (resistance determinant)[a]	Selecting drug	Frequency of mutation at:		
			2× MIC	4× MIC	8× MIC
1077	Macrolide S	CEM-101	$<9.1 \times 10^{-9}$	$<9.1 \times 10^{-9}$	$<9.1 \times 10^{-9}$
		Azithromycin	7.2×10^{-9}	$<1.9 \times 10^{-10}$	$<1.9 \times 10^{-10}$
		Clarithromycin	1.1×10^{-9}	$<1.2 \times 10^{-10}$	$<1.2 \times 10^{-10}$
		Telithromycin	1.1×10^{-9}	$<5.5 \times 10^{-10}$	$<5.5 \times 10^{-10}$
		Clindamycin	$<3.8 \times 10^{-10}$	$<3.8 \times 10^{-10}$	$<3.8 \times 10^{-10}$
24	Macrolide R [*erm*(B)]	CEM-101	1.8×10^{-7}	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$
		Azithromycin	NT[b]	NT	NT
		Clarithromycin	NT	NT	NT
		Telithromycin	1.3×10^{-4}	2.5×10^{-6}	1.7×10^{-6}
		Clindamycin	NT	NT	NT
3665	Macrolide R [*mef*(A)]	CEM-101	6.8×10^{-7}	1.4×10^{-7}	$<4.5 \times 10^{-10}$
		Azithromycin	NT	NT	NT
		Clarithromycin	5.0×10^{-7}	$<5.0 \times 10^{-10}$	$<5.0 \times 10^{-10}$
		Telithromycin	7.5×10^{-9}	2.5×10^{-9}	$<2.5 \times 10^{-10}$
		Clindamycin	$<2.4 \times 10^{-10}$	$<2.4 \times 10^{-10}$	$<2.4 \times 10^{-10}$
1076	Macrolide R [*erm*(B) *mef*(A)]	CEM-101	$<2.5 \times 10^{-8}$	7.5×10^{-9}	2.0×10^{-9}
		Azithromycin	NT	NT	NT
		Clarithromycin	NT	NT	NT
		Telithromycin	6.5×10^{-6}	9.7×10^{-6}	4.8×10^{-6}
		Clindamycin	NT	NT	NT
1635	Macrolide R [*erm*(A)]	CEM-101	1.6×10^{-8}	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$
		Azithromycin	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$
		Clarithromycin	$<3.1 \times 10^{-9}$	$<3.1 \times 10^{-9}$	$<3.1 \times 10^{-9}$
		Telithromycin	$<2.7 \times 10^{-9}$	$<2.7 \times 10^{-9}$	$<2.7 \times 10^{-9}$
		Clindamycin	7.3×10^{-7}	5.5×10^{-7}	5.6×10^{-7}
2686	Macrolide R (L4 mutation)	CEM-101	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$
		Azithromycin	NT	NT	NT
		Clarithromycin	NT	NT	NT
		Telithromycin	2.2×10^{-5}	2.2×10^{-9}	$<1.1 \times 10^{-9}$
		Clindamycin	$<1.2 \times 10^{-9}$	$<1.2 \times 10^{-9}$	$<1.2 \times 10^{-9}$
7127	Macrolide S (S20N in L4, A105V in L22)	CEM-101	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$
		Azithromycin	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$
		Clarithromycin	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$
		Telithromycin	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$
		Clindamycin	$<2.9 \times 10^{-8}$	$<2.9 \times 10^{-8}$	$<2.9 \times 10^{-8}$
3009	Macrolide R (23S rRNA mutation)	CEM-101	$<5.9 \times 10^{-9}$	$<5.9 \times 10^{-9}$	$<5.9 \times 10^{-9}$
		Azithromycin	NT	NT	NT
		Clarithromycin	NT	NT	NT
		Telithromycin	3.8×10^{-7}	$<1.5 \times 10^{-10}$	$<1.5 \times 10^{-10}$
		Clindamycin	1.7×10^{-4}	7.3×10^{-9}	$<1.2 \times 10^{-10}$

[a] S, susceptible; R, resistant.
[b] NT, not tested.

tant selection frequencies for CEM-101 ranged from $<5.9 \times 10^{-11}$ to 5.3×10^{-8} at 2× MIC to $<5.9 \times 10^{-11}$ to $<5.3 \times 10^{-10}$ at 8× MIC. The following comparators had higher frequencies of resistance than CEM-101: clindamycin, $<7.7 \times 10^{-11}$ to 2.1×10^{-7} at 2× MIC to $< 7.7 \times 10^{-11}$ to 1.1×10^{-7} at 8× MIC; clarithromycin, $<1.0 \times 10^{-10}$ to 1.7×10^{-7} at 2× MIC to $<1.0 \times 10^{-10}$ to 5.0×10^{-9} at 8× MIC. Mutant selection frequencies for telithromycin were similar to those for CEM-101: $<8.3 \times 10^{-11}$ to 7.7×10^{-8} at 2× MIC to $<8.3 \times 10^{-11}$ to $<6.3 \times 10^{-10}$ at 8× MIC. The mutation frequency for the one macrolide-sensitive strain tested with azithromycin was $<1.0 \times 10^{-10}$ at 2× and 8× MIC.

DISCUSSION

CEM-101 (Fig. 1) is a novel fluoroketolide that demonstrates enhanced potency compared to telithromycin, with activity against telithromycin-intermediate and telithromycin-resistant organisms (11). CEM-101 has shown significantly greater potency against phagocytized *Staphylococcus aureus* than telithromycin, azithromycin, and clarithromycin; CEM-101 was also about 50-fold and 100-fold more potent than azithromycin against phagocytized *Listeria monocytogenes* and *Legionella pneumophila* (17). CEM-101 exhibits the widest spectrum of activity against respiratory tract pathogens, includ-

TABLE 8. *S. pyogenes* single-step mutation frequencies

Strain no.	Phenotype (resistance determinant)[a]	Selecting drug	Frequency of mutation at:		
			2× MIC	4× MIC	8× MIC
2132	Macrolide S	CEM-101	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
		Azithromycin	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
		Clarithromycin	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
		Telithromycin	$<8.3 \times 10^{-11}$	$<8.3 \times 10^{-11}$	$<8.3 \times 10^{-11}$
		Clindamycin	$<7.7 \times 10^{-11}$	$<7.7 \times 10^{-11}$	$<7.7 \times 10^{-11}$
2368	Macrolide R [*erm*(B)]	CEM-101	$<5.9 \times 10^{-11}$	$<5.9 \times 10^{-11}$	$<5.9 \times 10^{-11}$
		Azithromycin	NT[b]	NT	NT
		Clarithromycin	NT	NT	NT
		Telithromycin	NT	NT	NT
		Clindamycin	NT	NT	NT
2094	Macrolide R [*erm*(A)]	CEM-101	5.3×10^{-8}	2.1×10^{-9}	$<5.3 \times 10^{-10}$
		Azithromycin	NT	NT	NT
		Clarithromycin	1.7×10^{-7}	1.0×10^{-7}	5.0×10^{-9}
		Telithromycin	7.7×10^{-8}	$<1.5 \times 10^{-10}$	$<1.5 \times 10^{-10}$
		Clindamycin	2.1×10^{-7}	1.3×10^{-7}	1.1×10^{-7}
2011	Macrolide R [*mef*(A)]	CEM-101	3.9×10^{-8}	$<1.1 \times 10^{-10}$	$<1.1 \times 10^{-10}$
		Azithromycin	NT	NT	NT
		Clarithromycin	NT	NT	NT
		Telithromycin	3.8×10^{-8}	$<6.3 \times 10^{-10}$	$<6.3 \times 10^{-10}$
		Clindamycin	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
237	Macrolide-R (L4 mutation)	CEM-101	$<1.3 \times 10^{-10}$	$<1.3 \times 10^{-10}$	$<1.3 \times 10^{-10}$
		Azithromycin	NT	NT	NT
		Clarithromycin	$<3.3 \times 10^{-10}$	$<3.3 \times 10^{-10}$	$<3.3 \times 10^{-10}$
		Telithromycin	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$
		Clindamycin	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$

[a] S, susceptible; R, resistant.
[b] NT, not tested.

ing multidrug-resistant pneumococcus type 19A, compared to azithromycin, clarithromycin, erythromycin, telithromycin, clindamycin, and quinupristin-dalfopristin (11). CEM-101 is also potent against *Chlamydia trachomatis*, *Chlamydophila pneumoniae* (27), human mycoplasmas, and ureaplasmas (29), and MICs also point to clinical utility against most enterococci, gonococci, and Gram-positive anaerobes (1). CEM-101 is active against common organisms that cause gastroenteritis, such as *Campylobacter jejuni*, *Salmonella* spp., and *Shigella* spp., and is also active against *Helicobacter pylori* (12). CEM-101 has been shown to be more bactericidal against several Gram-positive species than telithromycin, with postantibiotic effects (in hours) of 2.3 to 6.1 and 3.7 to 5.3 against Gram-positive and -negative strains, respectively (10). CEM-101 has also demonstrated significant in vivo activity in a variety of murine infection models (20). Preliminary multistep studies have shown CEM-101 to have no or modest variation in MICs for one strain each of *S. aureus* and *Enterococcus faecalis*, and for two *S. pneumoniae* strains; low rates of spontaneous mutants were found in single-step experiments (13). At a projected human therapeutic dose of 400 to 500 mg, a T_{max} (time to maximum concentration of the drug in serum) of 4 h and a $t_{1/2}$ (half-life) of 5 to 6 h would be expected for CEM-101. Increases in the maximum concentration of the drug in serum (C_{max}) and in the AUC were more than dose-proportional across the dose range administered (28).

In the current studies, CEM-101 yielded MICs that were usually a few dilutions lower than those of telithromycin against all resistance phenotypes of *S. pneumoniae* and *S. pyogenes* tested, including drug-resistant pneumococcus type 19A and *erm*(*B*)-positive *S. pyogenes*. Our results confirm previous findings cited above (11). CEM-101 yielded clones with higher MICs for all eight pneumococcal strains, but seven of the eight strains had clones with CEM-101 MICs of ≤0.5 μg/ml, and only for one *erm*(B) *mef*(A) strain with a parental MIC of 1 μg/ml was a resistant clone found with a MIC of 32 μg/ml. For two of the three resistant *S. pyogenes* CEM-101 clones [parental strains had the *erm*(A) gene or L4 mutations], MICs were 0.25 μg/ml, and only for the one strain with *erm*(B) did CEM-101 MICs rise from 1 to 8 μg/ml. Single-step studies also showed low yields of spontaneous mutations compared to those with other agents tested.

Based on pharmacokinetic findings reported from phase I clinical trials (28), recommendations for tentative CEM-101 susceptibility breakpoints have been set at ≤1 μg/ml as susceptible and ≥4 μg/ml as resistant; the tentative susceptibility breakpoint of ≤1 μg/ml is the same as that for telithromycin against streptococci (4).

The potent activity of CEM-101, and its low tendency to select for resistant mutants, against all streptococcal strains tested, irrespective of resistance phenotype, points to a promising clinical future for this compound, subject to pharmacokinetic/pharmacodynamic, toxicity, and animal infection model studies.

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