Antimicrobial Activity of Solithromycin against Serotyped Macrolide-Resistant *Streptococcus pneumoniae* Isolates Collected from U.S. Medical Centers in 2012

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Solithromycin, a next-generation macrolide and novel fluoroketolide, was tested against a 2012 collection of serotyped U.S. macrolide-resistant *Streptococcus pneumoniae* isolates associated with community-acquired bacterial pneumonia (CABP). Against all 272 isolates, solithromycin demonstrated high potency (MIC_{50/90}, 0.06/0.25 µg/ml), and it inhibited all strains at MICs of ≤0.5 µg/ml, including the two most prevalent macrolide-resistant serotypes (19A and 35B). These data support the continued clinical development of solithromycin for the treatment of multidrug-resistant CABP.

Worldwide, *Streptococcus pneumoniae* is the predominant causative agent of community-acquired bacterial pneumonia (CABP) and is an important pathogen in bacteremia, meningitis, and otitis media. In 2000, the seven-valent pneumococcal conjugate vaccine (PCV7) was introduced into the U.S. childhood vaccine schedule, followed by the PCV13 in 2010. Although the use of PCV7 was accompanied by marked reductions in invasive pneumococcal disease (IPD) (1), serotype replacement and the selective pressure of antimicrobial use have been associated with the emergence of multidrug-resistant (MDR) strains outside the spectrum of PCV7 vaccine coverage (such as MDR serotype 19A) (2–4).

Ketolides are semisynthetic antimicrobial agents derived from erythromycin A and were designed primarily to overcome macrolide-resistant streptococci, including MDR *S. pneumoniae*. Solithromycin (formerly CEM-101) is a next-generation oral and intravenous fluoroketolide in phase III clinical development for the treatment of moderate to moderate-severe CABP. Solithromycin has demonstrated potent activity against *S. pneumoniae*, including MDR and macrolide-resistant strains and genotypes (5, 6).

In this study, we report solithromycin and comparator activities, measured by reference Clinical and Laboratory Standards Institute (CLSI) standards, against 272 macrolide-resistant and serotyped clinical isolates collected in U.S. medical centers during 2012.

A total of 272 macrolide-resistant (erythromycin MIC, ≥1 µg/ml, and hence also resistant to azithromycin and clarithromycin by the CLSI interpretive criteria; MIC_{50/90}, 16/>16 µg/ml; Table 1) *S. pneumoniae* isolates collected during 2012 from 49 medical centers (in 35 states) across the United States were included (SENTRY Antimicrobial Surveillance Program). The isolates were recovered from lower respiratory tract specimens (82.7%) and blood cultures (17.3%) from patients across all age groups who had a diagnosis of CABP. The isolates were collected and submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, IA, USA) as part of the SENTRY Antimicrobial Surveillance Program. Bacterial identification was per-

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TABLE 1 Cumulative MIC distributions by serotype of solithromycin tested against macrolide-resistant *S. pneumoniae* isolates, United States, 2012

Serotype (no. of strains)	No. (cumulative %) inhibited at solithromycin MIC (µg/ml) of:					Solithromycin		Erythromycin	
	≤0.03	0.06	0.12	0.25	0.5	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀
19A (68)	5 (7.4)	6 (16.2)	20 (45.6)	35 (97.1)	2 (100.0)	0.25	0.25	>16	>16
35B (34)	6 (17.7)	9 (44.1)	11 (76.5)	7 (97.1)	1 (100.0)	0.12	0.25	16	>16
15B/15C (28)	24 (85.7)	2 (92.9)	2 (100.0)			≤0.03	0.06	4	16
6C/6D (26)	17 (65.4)	7 (92.3)	2 (100.0)			≤0.03	0.06	4	>16
15A/15F (23)	22 (95.7)	0 (95.7)	1 (100.0)			≤0.03	≤0.03	>16	>16
11A/11D (16)	3 (18.8)	0 (18.8)	10 (81.3)	3 (100.0)		0.12	0.25	8	>16
33F/33A/37 (12)	8 (66.7)	1 (75.0)	3 (100.0)			≤0.03	0.12	4	8
23A (11)	8 (72.7)	0 (72.7)	2 (90.9)	1 (100.0)		≤0.03	0.12	>16	>16
Other (54) ^a	17 (31.5)	12 (53.7)	13 (77.8)	11 (98.2)	1 (100.0)	0.06	0.25	8	>16
Total (272)	110 (40.4)	37 (54.0)	64 (77.6)	57 (98.5)	4 (100.0)	0.06	0.25	16	>16

^a Includes the following serotypes/serogroups (number of isolates): 3 (5), 8 (2), 13 (1), 14 (1), 20 (3), 31 (4), 34 (2), 10A (1), 12F/12A (5), 16F (1), 17F (2), 19F (8), 22F/22A (5), 23B (3), 23F (2), 6A/6B (2), 7C/7B (1), 7F/7A (2), 9N/9L (1), 9V/9A (1), and nontypeable (2).

TABLE 2 Antimicrobial susceptibilities by serotype of macrolide-resistant *S. pneumoniae* isolates collected in the United States during the 2012 SENTRY Antimicrobial Surveillance Program

Serotype (total no. of isolates/% tested)	% susceptible/intermediate/resistant ^a				
	Penicillin ^b	Ceftriaxone ^c	Clindamycin	Levofloxacin	T-S ^d
19A (68/25.0)	2.9/5.9/91.2	32.4/58.8/8.8	16.2/1.5/82.3	98.5/1.5/0.0	0.0/0.0/100.0
35B (34/12.5)	2.9/14.7/82.4	97.1/2.9/0.0	97.1/0.0/2.9	100.0/0.0/0.0	73.5/14.7/11.8
15B/15C (28/10.3)	25.0/75.0/0.0	100.0/0.0/0.0	92.8/3.6/3.6	100.0/0.0/0.0	21.4/75.0/3.6
6C/6D (26/9.6)	19.2/65.4/15.4	100.0/0.0/0.0	92.3/0.0/7.7	100.0/0.0/0.0	19.2/0.0/80.8
15A/15F (23/8.5)	0.0/91.3/8.7	100.0/0.0/0.0	0.0/0.0/100.0	100.0/0.0/0.0	52.2/17.4/30.4
11A/11D (16/5.9)	93.8/0.0/6.2	93.8/6.2/0.0	93.8/0.0/6.2	100.0/0.0/0.0	75.0/0.0/25.0
33F/33A/37 (12/4.4)	100.0/0.0/0.0	100.0/0.0/0.0	100.0/0.0/0.0	100.0/0.0/0.0	0.0/83.3/16.7
23A (11/4.0)	18.2/81.8/0.0	100.0/0.0/0.0	36.4/0.0/63.6	100.0/0.0/0.0	90.9/9.1/0.0
Other (54/19.8) ^e	64.8/18.5/16.7	88.9/9.3/1.8	75.9/0.0/24.1	100.0/0.0/0.0	63.0/13.0/24.0
Total (272/100.0)	29.0/32.0/39.0	80.1/17.3/2.6	61.0/0.7/38.3	96.4/0.4/0.0	38.2/17.7/44.1

^a Breakpoint criteria according to CLSI (8).^b Criteria as published by the CLSI (8) for "oral penicillin V" (susceptible [S], ≤ 0.06 $\mu\text{g/ml}$; intermediate [I], 0.12 to 1 $\mu\text{g/ml}$; resistant [R], ≥ 2 $\mu\text{g/ml}$).^c Criteria as published by the CLSI (8) for "ceftriaxone (nonmeningitis)" (S, ≤ 1 $\mu\text{g/ml}$; I, 2 $\mu\text{g/ml}$; R, ≥ 4 $\mu\text{g/ml}$).^d T-S, trimethoprim-sulfamethoxazole.^e Includes the following serotypes/serogroups (number of isolates): 3 (5), 8 (2), 13 (1), 14 (1), 20 (3), 31 (4), 34 (2), 10A (1), 12F/12A/44/46 (5), 16F (1), 17F (2), 19F (8), 22F/22A (5), 23B (3), 23F (2), 6A/6B (2), 7C/7B/40 (1), 7F/7A (2), 9N/9L (1), 9V/9A (1), and nontypeable (2).

formed by the participating microbiology laboratory and confirmed by the central monitoring laboratory (JMI Laboratories). Confirmation of the bacterial identification was performed by colony morphology and biochemical algorithms. When the bacterial identification was questionable using phenotypic methods or when an untypeable serotyping result was obtained, the isolates were subjected to a PCR assay for further identification.

The isolates were tested for susceptibility by broth microdilution methods, according to the recommendations of the CLSI (7). The MIC results for several anti-Gram-positive agents were obtained using panels manufactured by Thermo Fisher Scientific (Cleveland, OH, USA). Validation of the MIC values was performed by concurrent testing of the quality control (QC) strain *S. pneumoniae* ATCC 49619. In addition, the inoculum density was monitored by colony counts to ensure an adequate number of cells were present for each testing event. The MIC interpretations were based on the CLSI breakpoint criteria (8).

For serotype determination, PCR amplification of the *cpsB* gene was performed (9). Both strands of amplicons were sequenced, and the nucleotide sequences were analyzed using the Lasergene software package (DNASTar, Madison, WI) and compared to the sequence data available via BLAST (<http://www.ncbi.nlm.nih.gov/blast/>). Certain serotypes demonstrated sequence homology, and those showing similarities of $>99\%$ were grouped together (e.g., serotypes 9V/9A, 7F/7A, 11A/11D, 15A/15F, 22F/22A, 15B/15C). All serogroup 6 isolates underwent further PCR assays for confirmation and discrimination between 6A/6B and 6C/6D (10). The isolates that serogrouped as 6A/6B and 7F/7A were serotyped by the capsular swelling (Quellung) method using antisera, according to the manufacturer's instructions (Statens Serum Institut, Copenhagen, Denmark) (2, 11).

In total, 29 serotypes/serogroups were represented in this macrolide-resistant population of *S. pneumoniae*. Against all 272 isolates, solithromycin demonstrated high potency ($\text{MIC}_{50/90}$, 0.06/0.25 $\mu\text{g/ml}$) compared to that of erythromycin ($\text{MIC}_{50/90}$, 16/ >16 $\mu\text{g/ml}$), and it inhibited all strains at MIC values of ≤ 0.5 $\mu\text{g/ml}$ (Table 1). Although the potency remained high, solithromycin activity was slightly lower against the two most preva-

lent serotypes, 19A ($\text{MIC}_{50/90}$, 0.25/0.25 $\mu\text{g/ml}$) and 35B ($\text{MIC}_{50/90}$, 0.12/0.25 $\mu\text{g/ml}$), and against 11A/11D ($\text{MIC}_{50/90}$, 0.12/0.25 $\mu\text{g/ml}$) than against other serotypes and the overall population (Table 1).

Penicillin resistance, according to the CLSI oral penicillin V criteria (≥ 2 $\mu\text{g/ml}$), was high overall (39.0%) and extremely high in serotype 19A (91.2%) and 35B (82.4%) isolates. Intermediate susceptibility to penicillin was also high, at 32.0% overall. Ceftriaxone nonsusceptibility (≥ 2 $\mu\text{g/ml}$, according to CLSI nonmeningitis criteria) was 19.9% overall and very high in serotype 19A (67.6%) but not serotype 35B (2.9%) isolates. The clindamycin and trimethoprim-sulfamethoxazole nonsusceptibility rates were high, at 39.0% and 61.8%, respectively, and varied widely between serotypes/serogroups (Table 2). Most isolates (96.4%) were susceptible to levofloxacin.

In summary, solithromycin demonstrated potent activity against a geographically diverse set of macrolide-resistant *S. pneumoniae* isolates obtained in 2012 from patients with CABP across the United States. Solithromycin was very active against the two most prevalent macrolide-resistant serotypes (19A and 35B), in addition to the other prevalent serotypes/serogroups present in the overall population. These data support and encourage the continued clinical development of solithromycin for the treatment of multidrug-resistant community-acquired bacterial pneumonia.

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