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Antimicrobial Activity of Solithromycin Tested Against Serotyped Macrolide-resistant *Streptococcus pneumoniae* Collected from Medical Centers Across the USA (2012)

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Abstract

Objective: To evaluate the activity of solithromycin, a fourth generation macrolide and a novel fluoroketolide, tested against a contemporary (2012) collection of serotyped United States (USA) macrolide-resistant *S. pneumoniae* isolates associated with community-acquired bacterial pneumonia. Solithromycin was designed to overcome macrolide-resistant *S. pneumoniae.* With the introduction of new pneumococcal conjugate vaccines, the serotype distribution of *S. pneumoniae* has been dynamic in recent years, and hence monitoring the activity of new agents against circulating serotypes is prudent.

Methods: A total of 272 macrolide-resistant (erythromycin MIC, ≥1 mg/L) *S. pneumoniae* collected during 2012 from 49 medical centers (35 states) across the USA were included (SENTRY Antimicrobial Surveillance Program). Isolates were recovered from lower respiratory tract specimens (82.7%) and blood cultures (17.3%) in patients across all age groups with a diagnosis of community-acquired bacterial pneumonia. Species identification was performed using biochemical test algorithms and/or PCR assays. Serotyping was performed by *cpsB* sequencing and multiplex PCR methodology. Susceptibility testing applied CLSI methods (M07-A9) and interpretations were performed using CLSI M100-S24 (2014) breakpoint criteria.

Results: Against all 272 isolates, solithromycin demonstrated high potency ($MIC_{50/90}$, 0.06/0.25 mg/L) and inhibited all strains at MIC values ≤0.5 mg/L. Although potency remained high, solithromycin activity was slightly lower against the two most prevalent serotypes - 19A ($MIC_{50/90}$, 0.25/0.25 mg/L) and 35B ($MIC_{50/90}$, 0.12/0.25 mg/L) - compared to other serotypes and the overall population. In total, 29 serotypes/serogroups were represented in this population. Penicillin resistance by CLSI oral penicillin V criteria (≥2 mg/L) was high overall (39.0%) and extremely high in serotype 19A (91.2%) and serotype 35B (82.4%) isolates. Ceftriaxone-nonsusceptibility (≥2 mg/L, CLSI non-meningitis criteria) was 19.9% overall and very high in serotype 19A (67.6%), but not serotype 35B (2.9%) isolates.

Conclusions: Solithromycin demonstrated sustained activity against a geographically diverse set of macrolide-resistant *S. pneumoniae* isolated from patients with CABP across the USA in 2012. Solithromycin was shown to be 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.

Introduction

Worldwide, *Streptococcus pneumoniae* is the major causative microorganism 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 USA childhood vaccine schedule, followed by the PCV13 in 2010. Although the use of vaccines has been accompanied by impressive reductions in invasive pneumococcal disease (IPD), serotype replacement and the selective pressure of antimicrobial use have resulted in the emergence of multidrug-resistant (MDR) strains outside of vaccine coverage (such as MDR serotype 19A).

Introduction-continued

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 moderately-severe CABP. Solithromycin has demonstrated potent activity against *S. pneumoniae*, including most MDR and macrolide-resistant strains.

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

Materials and Methods

Clinical isolates. A total of 272 macrolide-resistant (erythromycin MIC, ≥1 mg/L and hence also resistant to azithromycin and clarithromycin by CLSI interpretive criteria) *S. pneumoniae* collected during 2012 from 49 medical centers (35 states) across the USA were included. Isolates were recovered from lower respiratory tract specimens (82.7%) and blood cultures (17.3%) in patients across all age groups with a diagnosis of community-acquired bacterial pneumonia. Isolates were collected and submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA), as part of the SENTRY Antimicrobial Surveillance Program.

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

Antimicrobial susceptibility testing. Isolates were tested for susceptibility by broth microdilution methods, according to the recommendations of CLSI. MIC results for several anti-Gram-positive agents were obtained using panels manufactured by ThermoFisher Scientific (formerly TREK Diagnostics Systems/Sensititre; Cleveland, Ohio, USA). Validation of the MIC values was performed by concurrent testing of quality control (QC) strain *S. pneumoniae* American Type Culture Collection (ATCC) 49619. In addition, the inoculum density was monitored by colony counts to ensure an adequate number of cells for each testing event. MIC interpretations were based on the EUCAST breakpoint criteria.

Pneumococcal serotyping. Isolates were subjected to PCR assays for amplification of the *cpsB* gene. Amplicons were sequenced on both strands and the nucleotide sequences were analyzed using the Lasergene software package (DNASTAR, Madison, Wisconsin). Sequences were compared to others available via Pubmed (http://www.ncbi.nlm.nih.gov/blast/). Due to sequence homology among certain serotypes, those showing nucleotide sequence similarity greater than 99% were grouped (e.g. 9V/9A, 7F/7A, 11A/11D, 15A/15F, 22F/22A, 15B/15C). All isolates determined to be serogroup 6 by sequencing analysis were subjected to multiplex PCR assays for confirmation and discrimination between 6A/6B and 6C/6D. Isolates determined to be serogroup 6A/6B and 7F/7A were serotyped by the capsular swelling method using commercially available antisera according to manufacturer's instructions (Statens Serum Institut, Copenhagen, Denmark).

Results

- In total, 29 serotypes/serogroups were represented in this macrolide-resistant population of *S. pneumoniae.* Against all 272 isolates, solithromycin demonstrated high potency ($MIC_{50/90}$, 0.06/0.25 mg/L) and inhibited all strains at MIC values ≤0.5 mg/L (Table 1). Although potency remained high, solithromycin activity was slightly lower against the two most prevalent serotypes - 19A ($MIC_{50/90}$, 0.25/0.25 mg/L) and 35B ($MIC_{50/90}$, 0.12/0.25 mg/L) - and against 11A/11D ($MIC_{50/90}$, 0.12/0.25 mg/L) compared to other serotypes and the overall population (Table 1).
- Penicillin resistance by CLSI oral penicillin V criteria (≥2 mg/L) was high overall (39.0%) and extremely high in serotype 19A (91.2%) and serotype 35B (82.4%) isolates. Intermediate susceptibility to penicillin was also high at 32.0% overall. Ceftriaxone-nonsusceptibility (≥2 mg/L, CLSI non-meningitis criteria) was 19.9% overall and very high in serotype 19A (67.6%), but not serotype 35B (2.9%) isolates.
- Clindamycin and trimethoprim/sulfamethoxazole non-susceptibility rates were high at 40.0% and 61.8%, respectively, and varied widely between serotypes/serogroups (Table 2). Most isolates (96.4%) were susceptible to levofloxacin.

Conclusions

- Solithromycin demonstrated potent activity against a geographically diverse set of macrolide-resistant *S. pneumoniae* isolated from patients with CABP across the USA in 2012. 100% of isolates were susceptible to solithromycin using the tentative breakpoint of 1 mg/L.
- 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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Table 1. Cumulative MIC distributions by serotype of solithromycin tested against macrolide-resistant *Streptococcus pneumoniae*, USA 2012.

	Distribution (cumulative % inhibited) at solithromycin MIC (mg/L) of:						
Serotype (no. of strains)	≤0.03	0.06	0.12	0.25	0.5	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
35B (34)	6 (17.7)	9 (44.1)	11 (76.5)	7 (97.1)	1 (100.0)	0.12	0.25
15B/15C (28)	24 (85.7)	2 (92.9)	2 (100.0)			≤0.03	0.06
6C/6D (26)	17 (65.4)	7 (92.3)	2 (100.0)			≤0.03	0.06
15A/15F (23)	22 (95.7)	0 (95.7)	1 (100.0)			≤0.03	≤0.03
11A/11D (16)	3 (18.8)	0 (18.8)	10 (81.3)	3 (100.0)		0.12	0.25
33F/33A/37 (12)	8 (66.7)	1 (75.0)	3 (100.0)			≤0.03	0.12
23A (11)	8 (72.7)	0 (72.7)	2 (90.9)	1 (100.0)		≤0.03	0.12
Other (54)[a]	17 (31.5)	12 (53.7)	13 (77.8)	11 (98.2)	1 (100.0)	0.06	0.25
Total (272)	110 (40.4)	37 (54.0)	64 (77.6)	57 (98.5)	4 (100.0)	0.06	0.25

a. Includes serotypes/serogroups (n): 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), nontypeable (2)

Table 2. Antimicrobial susceptibility of macrolide-resistant *S. pneumoniae* serotypes collected in USA during the 2012 SENTRY Antimicrobial Surveillance Program.

Serotype	% susceptible / intermediate / resistant[a]				
(Total no. tested/%)	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 (2014).
b. Criteria as published by the CLSI (2014) for 'Penicillin oral penicillin V' (S ≤0.06, I = 0.12-1, R ≥2 mg/L).
c. Criteria as published by the CLSI (2014) for 'Ceftriaxone (nonmeningitis)' (S ≤1, I = 2, R ≥4 mg/L).
d. T/S, Trimethoprim/sulfamethoxazole.
e. Includes serotypes/serogroups (n): 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), nontypeable (2).