

## Abstract (Revised)

**Background.** Solithromycin is in clinical development in adults and children. *Haemophilus influenzae* has intermediate susceptibility to macrolides and is known to persist in middle ear infections and in the lung of COPD patients. The purpose of this study was to determine MICs and MBCs of solithromycin and comparator drugs for nontypeable and typeable strains of *H. influenzae*.

**Methods.** Strains were obtained from a study of nasopharyngeal carriage of *H. influenzae* conducted in children by S. I. Pelton between July 1, 2010 and 2014. Serotypes were determined by slide agglutination using individual and poly antisera. MICs of solithromycin and comparator drugs were performed using broth microdilution in *Haemophilus* Test Medium as recommended by CLSI M7-A8. For determinations of MBCs of solithromycin and azithromycin, a total of 20 strains (14 nontypeable strains and 6 typeable strains) were selected from all strains tested to represent the range of observed MICs. MBCs were performed as recommend in CLSI M26-A.

**Results.** Of 210 clinical strains of *H. influenzae*, 165 strains were nontypeable and 45 strains were typeable. Of the typeable strains, 23 were serotype b, 13 were serotype d, 6 were serotype f, 2 were serotype e and 1 was serotype a. The range of MICs and MIC<sub>90</sub> of solithromycin were 0.12-8 µg/ml and 2 µg/ml, respectively. The mode MIC of solithromycin for nontypeable strains was 0.5 µg/ml and for typeable strains was 1 µg/ml. Comparing MIC<sub>90</sub>s for non-typeable strains, solithromycin was (1) at least two twofold dilutions more active than ampicillin, erythromycin and trimethoprim/sulfamethoxazole, (2) as active as amoxicillin/clavulanate and (3) one twofold dilution less active than azithromycin and cefdinir. For 17 of the 20 strains selected to represent the range of observed MICs, the MBCs of solithromycin were either equal to the MIC or only one twofold dilution greater than the MIC.

**Conclusions.** Solithromycin was active against *H. influenzae* isolates and was equally active against typeable and nontypeable strains. For 17 of the 20 strains of *H. influenzae*, solithromycin could be considered bactericidal. These results, and those previously presented in the chinchilla otitis media model could support future testing of solithromycin against *H. influenzae*-related otitis media.

## Introduction

*Haemophilus influenzae* is a gram-negative coccobacillus which can cause a wide variety of conditions such as pneumonia, bacteremia, meningitis, epiglottitis, otitis media, and bronchitis. *H. influenzae* can be characterized as either typeable or nontypeable based on whether or not the bacterium is encapsulated. If the bacterium is encapsulated, there are six possible serotypes (a-f) that it may possess. Typeable *H. influenzae* is often associated with invasive disease, such as pneumonia, especially serotype b. Non-typeable *H. influenzae* is commonly associated with otitis media in children, although it can also cause pneumonia<sup>1</sup>.

Solithromycin, a 4th generation macrolide, is in late clinical development for community-acquired bacterial pneumonia (CABP). It is expected to be available in oral, intravenous, and pediatric formulations. It has previously been shown to be effective in a chinchilla model of otitis media.

The purpose of this study was to determine minimum inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of solithromycin (CEM-101) and comparator drugs for nontypeable and typeable strains of *H. influenzae*.

## Methods

**Organisms.** Strains were obtained from a study of nasopharyngeal carriage of *H. influenzae* conducted in children (age 3 months to 5 years) by Stephen I. Pelton, M.D., Boston University Medical Center, Boston, MA, between July 1, 2010 and present. Serotyping of strains was determined by slide agglutination using individual and poly antisera specific to *H. influenzae* serotypes a, b, c, d, e and f (Difco® *Haemophilus influenzae* Antiserum; Becton, Dickinson and Company, Sparks, MD).

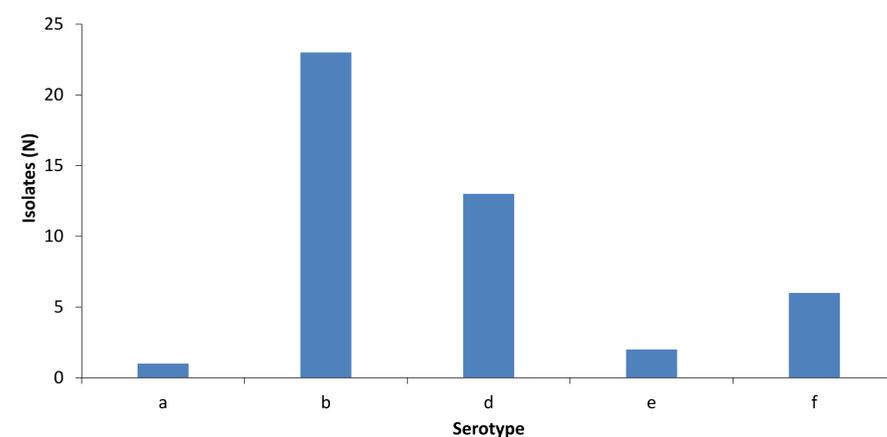
**Drugs.** MICs of the following drugs were determined: amoxicillin, ampicillin, azithromycin, cefdinir, CEM-101 (solithromycin), clavulanate, erythromycin, telithromycin, trimethoprim, sulfamethoxazole. Drugs were dissolved and diluted for testing per recommendations in CLSI A7-A8<sup>3</sup> and CLSI M100-S24<sup>4</sup>.

**MBC Determinations.** MBCs of solithromycin and azithromycin were determined for 20 strains (14 nontypeable strains and 6 typeable strains) selected to represent the range of observed MICs. 10 µl was removed in duplicate by pipet from microdilution wells containing one-half the MIC, the MIC and each concentration above the MIC and plated by spreading onto Chocolate Agar followed by incubation for 48 hours at 35°C in ambient air with 5% CO<sub>2</sub>. Following incubation, mean colony counts for each concentration plated were determined. The drug concentration which yielded a colony count representing ≤0.1% of the original inoculum (i.e., a 99.9% reduction in viability of the original inoculum as determined by actual colony counts from the growth control well) taking into account pipetting error and intrinsic sampling variability was considered the bactericidal concentration (CLSI M26-A<sup>5</sup>).

## Results

The distribution of serotyped *H. influenzae* are shown in Figure 1. *H. influenzae* type b was the most common serotype in this collection of strains.

**Figure 1. Distribution of *H. influenzae* serotypes (N=45)**



## Results (continued)

**Table 1. Activity against Non-Typeable and Typeable *H. influenzae***

MICs for Non-Typeable <i>H. influenzae</i> (N=165)			
	Range	MIC 50%	MIC 90%
Solithromycin	0.12 - 8	1	2
Telithromycin	0.12 - 16	1	2
Azithromycin	0.06 - 4	0.5	1
Erythromycin	0.12 - 16	2	8
Ampicillin	0.06 - >64	0.25	64
Amox/Clav	0.12/0.06 - 8/4	0.5/0.25	2/1
Cefdinir	≤0.03 - 2	0.25	1
Trim/Sulfa	≤0.015/0.3 - 32/608	0.03/0.6	8/152

MICs for Typeable <i>H. influenzae</i> (N=45)*			
	Range	MIC 50%	MIC 90%
Solithromycin	0.5 - 4	1	4
Telithromycin	0.5 - 4	1	2
Azithromycin	0.12 - 2	0.5	1
Erythromycin	1 - 16	4	8
Ampicillin	0.06 - 64	0.25	64
Amox/Clav	0.03/0.015 - 4/2	0.25/0.12	2/1
Cefdinir	0.06 - 1	0.25	1
Trim/Sulfa	≤0.015/0.3 - 32/608	0.06/1.19	8/152

\* 1 serotype a; 23 serotype b; 13 serotype d; 2 serotype e; 6 serotype f

Comparing MIC<sub>90</sub> values for non-typeable strains, solithromycin was:

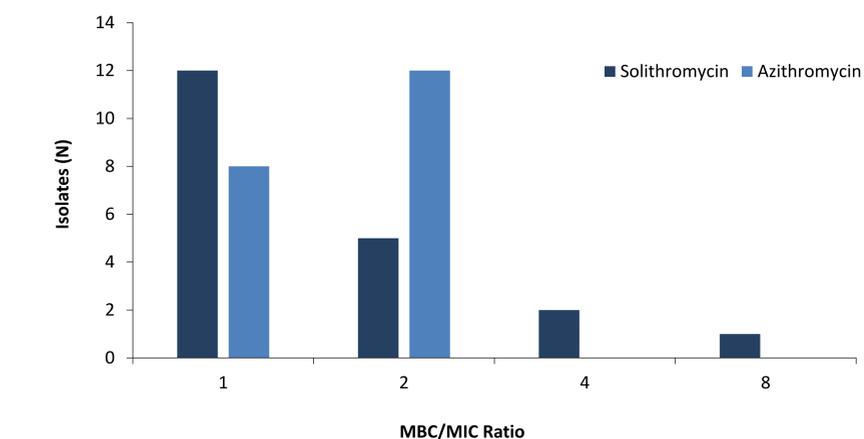
- at least two twofold dilutions more active than ampicillin, erythromycin and trimethoprim/sulfamethoxazole;
- as active as amoxicillin/clavulanate;
- one twofold dilution less active than azithromycin and cefdinir (Table 1).

The modal MIC of solithromycin for nontypeable strains was 0.5 µg/ml and for typeable strains was 1 µg/ml.

## References

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- Rodvold, K. A., M. H. Gotfried, J. G. Still, K. Clark, and P. Fernandes. 2012. Comparison of plasma, epithelial lining fluid, and alveolar macrophage concentrations of solithromycin (CEM-101) in healthy adult subjects. *Antimicrob. Agents Chemother.* 56:5076-5081.
- Still, J. G., J. Schranz, T. P. Degenhardt, D. Scott, P. Fernandes, M. J. Gutierrez, and K. Clark. 2011. Pharmacokinetics of solithromycin (CEM-101) after single or multiple oral doses and effects of food on single-dose bioavailability in healthy adult subjects. *Antimicrob. Agents Chemother.* 55:1997-2003.
- Azithromycin Prescribing Information.

**Figure 2. MBC/MIC Ratios against *H. influenzae***



- For 17 of 20 strains selected to represent the range of observed MICs, the MBCs of solithromycin were either equal to the MIC or only one twofold dilution greater than the MIC.
- For the 3 strains with higher MBC/MIC ratios, solithromycin MBCs ranged from 0.5 to 4 µg/mL, concentrations readily achieved in tissues<sup>6</sup>.
- The MBCs of azithromycin were either equal to the MIC or only one twofold dilution greater than the MIC.

## Conclusions

- Solithromycin was active against *H. influenzae* isolates and was equally active against typeable and nontypeable strains.
- For 17 of the 20 strains of *H. influenzae*, solithromycin could be considered bactericidal.
- Solithromycin has been reported to reach high plasma and tissue concentrations, higher than those reported for azithromycin<sup>6-8</sup>.
- These results, and those previously presented in the chinchilla otitis media model could support future testing of solithromycin against *H. influenzae*-related otitis media.