

Revised Abstract

Objectives: Solithromycin is a fourth generation macrolide, the first fluoroketolide, that is currently undergoing Phase III clinical development for the treatment of community-acquired bacterial pneumonia and is being developed as oral capsules, intravenous and pediatric suspension. This study evaluated the *in vitro* activity of solithromycin against respiratory streptococcal isolates tested by the Clinical Laboratory and Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution methods.

Methods: A total of 165 streptococci, 113 *S. pneumoniae* and 52 *S. pyogenes*, collected from Europe, Asia-Pacific and North America in 2012-13 were investigated. Minimum inhibitory concentration (MIC) for solithromycin was determined by broth microdilution in Mueller Hinton broth supplemented with 3% lysed horse blood (CLSI) and in Mueller Hinton broth supplemented with 5% defibrinated horse blood & 5 mg/L NAD (EUCAST).

Results: Very similar MIC values were obtained by either method. When CLSI MIC was plotted against EUCAST MIC the r^2 was 0.75 for *S. pneumoniae* and 0.79 for *S. pyogenes*. MIC distribution for combined streptococci is given in the Figure ($r^2 = 0.75$).

Solithromycin MIC distribution

Solithromycin MIC (mg/L)	CLSI MIC (mg/L) ≤0.001	CLSI MIC (mg/L) 0.002	CLSI MIC (mg/L) 0.004	CLSI MIC (mg/L) 0.008	CLSI MIC (mg/L) 0.015	CLSI MIC (mg/L) 0.03	CLSI MIC (mg/L) 0.06	CLSI MIC (mg/L) 0.12	CLSI MIC (mg/L) 0.25	CLSI MIC (mg/L) 0.5	CLSI MIC (mg/L) 1
0.50										2	3
0.25										4	
0.12								2	1	1	
0.06					1	6					
0.03	1	1	7	15	4	3					
0.015			7	18	3						
0.008			45	26	1						
0.004		1	9	1							
0.002		1		1							

Conclusions: Solithromycin showed very consistent activity against pneumococci and *S. pyogenes* using CLSI or EUCAST methods. Although susceptibility breakpoints are not yet available for solithromycin these data suggest that testing by either method in the future will be comparable.

References

1. Oikari D, Clark K, Sotiriou S, Das A, Craft JC, Scott D, Jamieson BD, Fernandes P. 2013. Randomised, double-blind, multicenter phase 2 study comparing the efficacy and safety of oral solithromycin (CEM-101) to those of oral levofloxacin in the treatment of patients with community-acquired bacterial pneumonia. *Antimicrob Agents Chemother*. 57:2026-34.
2. Clinical Laboratory Standards Institute. 2012. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. Approved Standards – Eighth Edition. CLS document M7-A8. Wayne, PA.
3. European Committee for Antimicrobial Susceptibility Testing. 2005. Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. EUCAST discussion document E.04 S.1. *Clin Micro Infect* 9:1-7.
4. Clinical Laboratory Standards Institute. 2014. *Performance Standards for Antimicrobial Susceptibility Testing*. Twenty Fourth Informational Supplement. CLS document M100-S24. Wayne, PA.

Acknowledgements

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Introduction

Solithromycin is a fluoroketolide available in both oral and intravenous formulations. It is being developed for the treatment of community-acquired bacterial pneumonia (CABP) and urethritis. Solithromycin is currently undergoing Phase 3 clinical trials for the treatment of moderate to moderately-severe CABP. Phase 2 clinical trial data showed solithromycin to be equivalent to levofloxacin in efficacy and to have a more favourable safety profile [1]. This study evaluated the *in vitro* activity of solithromycin against more than 165 respiratory streptococcal isolates collected in Europe and North America during 2012-2013 and compared results using Clinical Laboratory and Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution methods.

Materials and Methods

A total of 113 *S. pneumoniae* and 52 *S. pyogenes* were tested from Europe, Asia-Pacific and North America (Table 1). Isolates were identified to the species level and MICs determined at a central testing laboratory (IHMA Europe, located in Epalinges, Switzerland). These isolates are a sub-set of the clinical isolates presented in Poster P-1584. Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [2] and the EUCAST broth microdilution testing method using panels prepared at IHMA Europe [3]. Quality controls (QC) were performed on each day of testing using *S. pneumoniae* ATCC 49619 control strain. Approved solithromycin MIC ranges for the CLSI method are 0.004 to 0.015 mg/L [4]. To date, there are no published ranges for the EUCAST method.

Results

Repeat QC data for *S. pneumoniae* ATCC 49619 are given in Figure 1. One experiment was out of range for CLSI testing and two were out of range applying the same concentrations for EUCAST testing (MIC = 0.06 mg/L). These were all obtained on the same day of testing.

MIC distribution for solithromycin by both methods against *S. pneumoniae* and *S. pyogenes* are shown in Table 2. Scatter plots for *S. pneumoniae* and *S. pyogenes* are given in Figures 2 and 3, respectively.

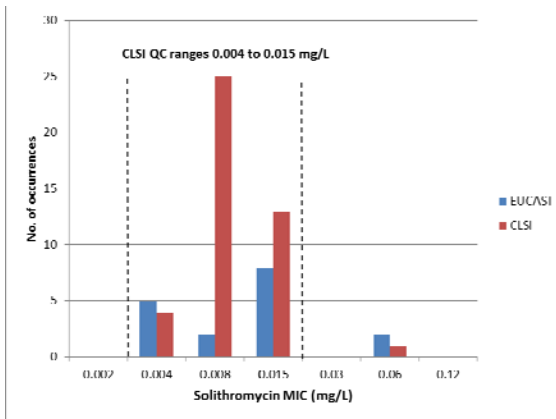
Table 1: Number of isolates investigated and region of origin.

Region	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pyogenes</i>
Asia	2	0
Europe	57	27
North America	54	25
Total	113	52

Table 2: MIC distribution for solithromycin tested against streptococci using CLSI or EUCAST broth microdilution methods.

Organism (N)	Method	Number of isolates at MIC (mg/L)											MIC ₅₀	MIC ₉₀
		≤0.001	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1		
<i>S. pneumoniae</i> (113)	CLSI	1	1	2	57	22	9	9	1	6	4	1	0.008	0.12
	EUCAST		2	11	47	8	26	7	2	4	6		0.008	0.12
<i>S. pyogenes</i> (52)	CLSI				12	38			1	1			0.015	0.015
	EUCAST				25	20	5		2				0.015	0.03

Figure 1: Repeat testing for QC strain *S. pneumoniae* ATCC 49619



Dotted line represents CLSI QC range. Number of occurrences are higher for CLSI as these were run as part of a larger study [P-1584 ECCMID 2014].

Figure 3: Scatter plot of CLSI vs EUCAST MIC for 52 *S. pyogenes*.

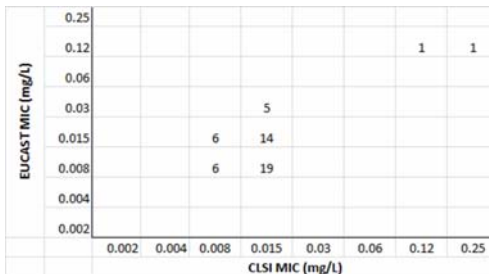
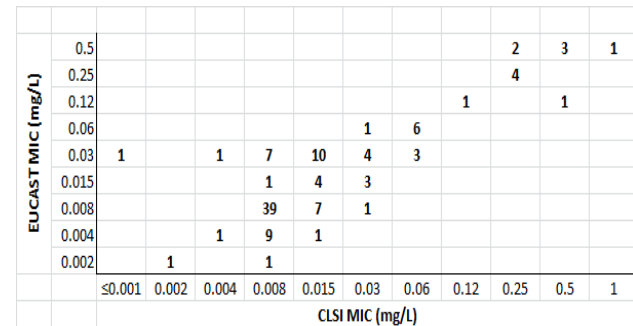


Figure 2: Scatter plot of CLSI vs EUCAST MIC for 113 *S. pneumoniae*.



Conclusions

- Solithromycin showed very consistent activity against pneumococci and *S. pyogenes* using CLSI or EUCAST methods.
- QC data using *S. pneumoniae* ATCC 49619 were also very reproducible using either method.
- These data show that solithromycin susceptibility testing by these two broth microdilution methods are comparable.