Activity of Solithromycin Against Azithromycin-Resistant *Streptococcus pneumoniae* with Characterized Resistance Mechanisms

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Revised Abstract

Objectives: Solithromycin is a fourth generation macrolide, the first fluoroketolide being developed in oral capsules, intravenous and pediatric suspension, that is currently undergoing Phase 3 clinical development for the treatment of community-acquired bacterial pneumonia. This study evaluated the *in vitro* activity of solithromycin against *S. pneumoniae* resistant to azithromycin and characterized the associated macrolide resistance mechanisms.

Methods: A total of 996 *S. pneumoniae* were collected from Europe, Asia-Pacific, North America and the rest of the world. Isolates were tested in a central laboratory with MIC and susceptibility for solithromycin and comparators determined according to CLSI broth microdilution methodology and breakpoints. Those isolates found to be resistant to azithromycin were evaluated for the presence of *erm*(A), *erm*(B), *erm*(C), *MsrA/B*, *ereA*, *ereB*, *mphA*, *mef*(A) and *mef*(E) genes by PCR.

Results: A total of 395 *S. pneumoniae* were found to be azithromycin-resistant (MIC >=2 mg/L) and 394 were available for molecular evaluation. Overall, the main genotypes were *erm*(B) (146, 37.1%), *erm*(B) & *mef*(E) (115, 29.2%) and *mef*(E) (77, 19.5%). Eight isolates (2.0%) were negative for all resistance genes and 33 (8.4%) gave inconclusive results. The dominant genotype was *erm*(B) in Europe (47.9%) and Asia-Pacific (53.8%), but *mef*(E) in North America (44.4%). Other mechanisms were found in fewer than 6 isolates each (data not shown). Solithromycin was more active against *erm*(B) isolates than *mef*(E) and least active against isolates with both mechanisms (see Figure 2 in main poster). Nevertheless, solithromycin MICs were no greater than 1 mg/L against all azithromycin-resistant isolates.

Conclusions: Solithromycin showed excellent activity against pneumococci resistant to azithromycin, particularly against strains with *erm*(B), which is the most common resistance mechanism world-wide. This strong potency was present even against isolates with multiple macrolide resistance mechanisms. These data support the continued development of solithromycin for the treatment of respiratory infections caused by pneumococci, even for those isolates resistant to macrolides.

Introduction

Solithromycin is a fluoroketolide available in both oral and intravenous formulation. 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 favorable safety profile [1].

This study evaluated the *in vitro* activity of solithromycin against molecularly-characterised azithromycin-resistant *Streptococcus pneumoniae* collected in Europe, Asia-Pacific, North America and the rest of the world during 2012-2013.

Materials & Methods

- A total of 996 *S. pneumoniae* were tested from Europe, Asia-Pacific, North America and the rest of the world (Table 1). Isolates were identified to the species level and MICs determined at a central testing laboratory (IHMA Europe, located in Epalinges, Switzerland).
- Minimum inhibitory concentrations (MICs) were determined by the Clinical and Laboratory Standards Institute (CLSI) recommended broth microdilution testing method using panels prepared at IHMA [2].
- MIC interpretive criteria mainly followed published guidelines of CLSI published in 2013 [3].
- Quality controls were performed on each day of testing using appropriate ATCC control strains, following CLSI and manufacturer guidelines. Results were included in the analysis only when corresponding QC results were within the acceptable ranges [3].
- Molecular characterization of macrolide-resistant pneumococci (presence of *erm*(A), *erm*(B), *erm*(C), *msrA/B*, *ereA*, *ereB*, *mphA*, *mef*(A) and *mef*(E) genes) was determined by PCR as described by Sutcliffe *et al* [4].

References

1. Oldach D, Clark K, Schranz J, Das A, Craft JC, Scott D, Jamieson BD, Fernandes P. 2013. Randomized, 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. 57:2526-34.
2. Clinical Laboratory Standards Institute. 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards – Ninth Edition. CLSI document M07-A9. Wayne, PA.
3. Clinical and Laboratory Standards Institute. 2013. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. CLSI Document M100-S23. Wayne, PA.
4. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. 1996. Detection of erythromycin-resistance determinants by PCR. Antimicrob Agents Chemother. 40:2562-6.

Acknowledgements

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Results

Susceptibility data for ketolides and macrolides are shown in Table 2. A total of 395 isolates were found to be resistant to azithromycin as shown in Table 3. Prevalence of macrolide resistance mechanisms in the 395 azithromycin-resistant isolates by region is shown in Figure 1. The cumulative MIC distributions for solithromycin against the three main resistance mechanisms observed are shown in Figure 2. Summary MIC data for solithromycin compared with telithromycin against the three main resistance mechanisms observed are shown in Table 4.

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

Region	*Streptococcus pneumoniae*
Europe	418
North America	380
Asia-Pacific	129
Latin America	32
Africa	20
Middle East	17
TOTAL	996

Table 2: Summary MIC and susceptibility for ketolides and macrolides against 996 *S. pneumoniae*.

Drug	CLSI Breakpoints (S|I|R)	Region	N	Percentage:			MIC (mg/L):			
				Susceptible	Intermediate	Resistant	MIC_{50}	MIC_{90}	Minimum	Maximum
Solithromycin	≤1 | 2 | >=4[a]	ALL	996	100	0.0	0.0	0.008	0.25	≤ 0.001	1
		Asia-Pacific	129	100	0.0	0.0	0.06	0.5	0.004	1
		Europe	418	100	0.0	0.0	0.008	0.06	≤ 0.001	0.5
		North America	380	100	0.0	0.0	0.008	0.25	0.002	0.5
		ROW[b]	69	100	0.0	0.0	0.008	0.5	0.002	0.5
Telithromycin	≤1 | 2 | >=4	ALL	996	99.5	0.5	0.0	0.015	0.5	≤ 0.002	2
		Asia-Pacific	129	99.2	0.8	0.0	0.06	0.5	0.008	2
		Europe	418	100	0.0	0.0	0.008	0.12	≤ 0.002	1
		North America	380	99.5	0.5	0.0	0.015	0.5	0.004	2
		ROW[b]	69	97.1	2.9	0.0	0.015	0.5	0.004	2
Azithromycin	≤0.5 | 1 | >=2	ALL	996	59.4	0.9	39.7	0.12	> 1	≤ 0.03	> 1
		Asia-Pacific	129	28.7	0.8	70.5	> 1	> 1	≤ 0.03	> 1
		Europe	418	71.3	0.7	28.0	0.12	> 1	≤ 0.03	> 1
		North America	380	56.3	1.1	42.6	0.12	> 1	≤ 0.03	> 1
		ROW[b]	69	62.3	1.5	36.2	0.12	> 1	≤ 0.03	> 1
Erythromycin	≤0.25 | 0.5 | >=1	ALL	996	60.1	0.3	39.6	≤ 0.06	> 0.5	≤ 0.06	> 0.5
		Asia-Pacific	129	29.5	0.0	70.5	> 0.5	> 0.5	≤ 0.06	> 0.5
		Europe	418	72.3	0.5	27.3	≤ 0.06	> 0.5	≤ 0.06	> 0.5
		North America	380	56.8	0.0	43.2	≤ 0.06	> 0.5	≤ 0.06	> 0.5
		ROW[b]	69	62.3	1.5	36.2	≤ 0.06	> 0.5	≤ 0.06	> 0.5

[a]provisional solithromycin breakpoints
[b]ROW = Latin America (32), Africa (20) & Middle East (17)

Table 3: Distribution of azithromycin-resistant and/or telithromycin-intermediate *S. pneumoniae* by region.

Region	Number of *S. pneumoniae*:		Total
	Azithromycin-resistant	Azithromycin-resistant and Telithromycin-intermediate	
North America	160	2	162
Europe*	117	0	117
Asia-Pacific	90	1	91
Latin America	9	0	9
Africa	7	1	8
Middle East	7	1	8
Grand Total	390	5	395

*One macrolide-resistant isolate from Europe was not available for molecular testing.

Table 4: Summary MIC data for solithromycin and telithromycin against the three main macrolide resistance mechanisms observed.

Resistance mechanism (N)	Solithromycin		Telithromycin	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
erm(B) (146)	0.03	0.25	0.03	0.25
erm(B) & *mef*(E) (115)	0.25	0.5	0.5	1
mef(E) (77)	0.06	0.25	0.25	0.5

Figure 2: Cumulative percentage MIC distribution for solithromycin against the three main macrolide resistance mechanisms observed.

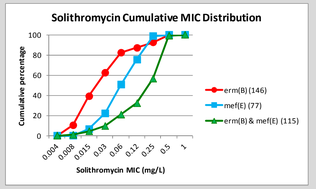
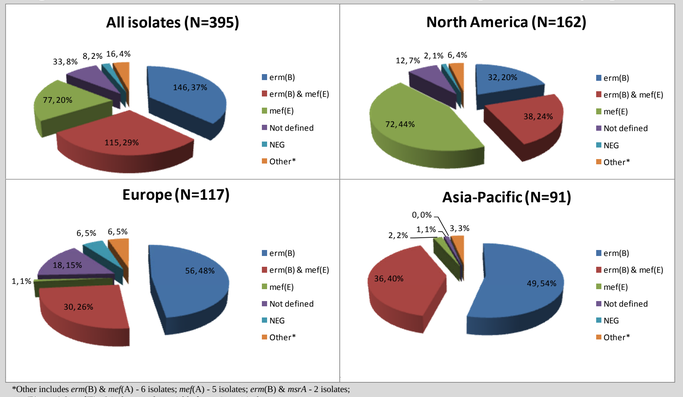


Figure 1: Prevalence of macrolide-resistance mechanisms in *S. pneumoniae* by region.



*Other includes *erm*(B) & *mef*(A) - 6 isolates; *mef*(A) - 5 isolates; *erm*(B) & *msrA* - 2 isolates; *erm*(B), *msrA* & *mef*(E) - 2 isolates; and not viable for testing - 1 isolate.

Conclusions

- Macrolide resistance in *S. pneumoniae* was ~40% overall, but much higher in Asia-Pacific (~70%) and lower in Europe (~28%).
- Overall *erm*(B) was the most common macrolide resistance mechanism (37%) followed by *erm*(B) & *mef*(E) combined (29%) and *mef*(E) at 20%. However, the majority of *mef*(E) were found in North America and this was the most prevalent mechanism in this region (44%)
- Solithromycin showed excellent activity against *S. pneumoniae* resistant to azithromycin, even against isolates with multiple macrolide resistance mechanisms.
- Solithromycin and telithromycin showed equal activity against pneumococci with *erm*(B) but solithromycin was more active against isolates that were *mef*(E)-positive [either alone or in combination with *erm*(B)]. This suggests that solithromycin may be a poor substrate for *mef*(E)-mediated efflux.
- These data support the continued development of solithromycin for the treatment of respiratory infections caused by pneumococci, even for those isolates from regions of the world with high macrolide resistance.