C-550

Assessment of the bactericidal activity of solithromycin (CEM-101) against *Streptococcus pneumoniae* with known macrolide-resistance mechanism and serotype

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Revised Abstract

Background: Solithromycin is a next-generation macrolide, an oral and intravenous fluoroketolide currently in clinical development for the treatment of community-acquired bacterial pneumonia (CABP). The current study investigated the bactericidal activity of solithromycin by determining minimum bactericidal concentration (MBC) against *Streptococcus pneumoniae* with known macrolide resistance mechanisms and serotype. **Methods:** A total of 33 clinical isolates of *S. pneumoniae* (8 azithromycin-susceptible and 25 azithromycin-resistant) were tested. These isolates included a diverse range of serotypes and macrolide resistance genotypes. MIC tests were performed by broth microdilution against all isolates in line with CLSI susceptibility testing standards (M07-A10). MBC was determined by sampling from the MIC plates as per CLSI guidelines (M26-A) and defined as the lowest concentration of antibacterial agent required to kill 99.9% of the test inoculum. **Results**: Summary MIC and MBC data for solithromycin and azithromycin as control are shown in the Table. Most isolates had a solithromycin MBC/MIC ratio of 4 or below (22/33, 66%), although one isolate had a ratio of 256. This isolate had a very low MIC of 0.004 µg/ml, so the MBC was still relatively low at 1 µg/ml. For most macrolide-resistant isolates the azithromycin MBC was beyond the limit of detection, so MBC/MIC ratio could not be determined.

	Phenotype	MIC (µg/ml):			MBC (µg/ml):		
		50%	90%	Range	50%	90%	Range
Azithromycin	AZI-R (n=25)	>256	>256	2 to >256	>256	>256	8 to >256
	AZI-S (n=8)	0.03	0.5	0.008 - 0.5	0.06	1	0.008 - 1
Solithromycin	AZI-R (n=25)	0.03	0.5	0.002 - 0.5	0.25	1	0.002 - 4
	AZI-S (n=8)	0.002	0.015	0.002 -0.015	0.004	0.06	0.004 - 0.06

AZI-S, azithromycin-susceptible; AZI-R, azithromycin-resistant

Conclusions: Solithromycin exhibited superior MIC and MBC as compared with azithromycin. Importantly, MBC/MIC ratios for solithromycin were favourable. The ratio of MBC/MIC for solithromycin did not appear to relate to solithromycin MIC, genotype or serotype.

Introduction

Solithromycin is a next-generation oral and intravenous fluoroketolide currently in clinical development for the treatment of community-acquired bacterial pneumonia (CABP). The current study investigated the bactericidal activity of solithromycin by determining minimum bactericidal concentration (MBC) against *Streptococcus pneumoniae* previously characterized for macrolide resistance mechanisms.

Materials & Methods

Isolates. A total of 33 recent clinical isolates of *S. pneumoniae* were used in this study and these originated from various countries worldwide.

Serotyping. *S. pneumoniae* were serotyped using multiplex PCR assays that detect serotype-specific genes within the capsular polysaccharide synthesis (*cps*) operon of *S. pneumoniae* to detect serotypes 1, 3, 4, 6A/B, 7C/B, 7F/A, 8, 9V/A, 10A, 11A/D, 12F/A, 14, 15A, 15B/C, 16F, 17F, 18A/B/C/F, 19A, 19F, 20, 22F/A, 23F, 31, 33F/A, 34, 35B, 35F and 38 as described by Hackel *et al* [1]. Isolates that could not be typed definitively by PCR were serotyped using the conventional Quellung reaction using type-specific antisera (Statens Serum Institute, Copenhagen, Denmark).

Genotyping. Azithromycin-resistant isolates were investigated for the presence of *ermA*, *ermB*, *ermC*, *MsrA/B*, *ereA*, *ereB*, *mphA* and *mefA/E* by PCR as described by Sutcliffe et al [2].

MIC determination. All isolates were tested for MIC by broth microdilution in Mueller Hinton broth supplemented with 5% lysed horse blood in line with Clinical and Laboratory Standards Institute (CLSI) methodology [3] using frozen 96-well MIC panels prepared at IHMA. Susceptibility to azithromycin was determined according to CLSI breakpoints [4]

MBC determination. Post-MIC incubation, two aliquots of 10 µl were taken from each well of the MIC plate where visible growth was not observed and sub-cultured onto Mueller Hinton agar plates supplemented with 5% lysed horse blood. These plates were tilted to spread the inoculum and then allowed to dry before being incubated for 20 to 24 h at 35 °C in 5% CO_2. Colonies were counted and MBC determined according to the CLSI guideline M26-A [4]. MBC was defined as the lowest concentration of antibacterial agent required to kill 99.9% (3 x $\log_{10}$) of the test inoculum [5].

Results

- **The isolates tested included a wide range of different serotypes (Figure 1) and genotypes (Figure 2).**
- **Solithromycin MIC and MBC was unrelated to serotype or genotype (data not shown).**
- **Solithromycin had lower MICs (Table 1) and MBCs (Table 2) than azithromycin.**
- **Almost 50% of isolates had azithromycin MBCs >256 µg/ml whereas the maximum solithromycin MBC was 4 µg/ml (Table 2).**
- **The ratio of solithromycin MBC to MIC was unrelated to solithromycin MIC (Table 3); most isolates (66%) had a ratio of 4 or lower.**

Table 1 MIC distributions for azithromycin and solithromycin against azithromycin-susceptible and -resistant *S. pneumoniae*

AZI	MIC (µg/ml):																N	MIC (µg/ml):	
	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>256		50%	90%
AZI-R										1	3	4	2	1			14	25	>256	>256
AZI-S			2	1	1	3			1									8	0.03	0.5
ALL			2	1	1	3			1		1	3	4	2	1		14	33	16	>256

SOL	MIC (µg/ml):																N	MIC (µg/ml):	
	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>256		50%	90%
AZI-R	2	1	3		7	3	4	2	3									25	0.03	0.5
AZI-S	7			1														8	0.002	0.015
ALL	9	1	3	1	7	3	4	2	3									33	0.03	0.25

AZI, azithromycin; SOL, solithromycin; -S, susceptible; -R, resistant

Table 2 MBC distributions for azithromycin and solithromycin against azithromycin-susceptible and -resistant *S. pneumoniae*

AZI	MBC (µg/ml):																N	MBC (µg/ml):	
	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>256		50%	90%
AZI-R												3	1	4	1	16		25	>256	>256
AZI-S			1	1	1	4				1								8	0.06	1
ALL			1	1	1	4				1		3	1	4	1	16		33	64	>256

SOL	MBC (µg/ml):																N	MBC (µg/ml):	
	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>256		50%	90%
AZI-R	1			1	1	3	3	5	3	6	1	1						25	0.25	1
AZI-S		4		2	1	1												8	0.004	0.06
ALL	1	4		3	2	4	3	5	3	6	1	1						33	0.12	1

AZI, azithromycin; SOL, solithromycin; -S, susceptible; -R, resistant

Table 3 Ratio of solithromycin MBC/MIC for all *S. pneumoniae* (n=33) by solithromycin MIC.

Solithromycin MIC (µg/ml)	MBC/MIC ratio							Grand Total
	1	2	4	8	16	32	256	
0.002	5	2		2				9
0.004							1	1
0.008			1	1	1			3
0.015		1						1
0.03		2	1	2	1	1		7
0.06		1	2					3
0.12		1	1	1		1		4
0.25		1	1					2
0.5		2	1					3
Grand Total (N)	**5**	**10**	**7**	**6**	**2**	**2**	**1**	**33**
Cumulative %	**15%**	**45%**	**66%**	**85%**	**91%**	**97%**	**100%**	

Figure 1. Serotype distribution for the *S. pneumoniae* investigated (n, %)

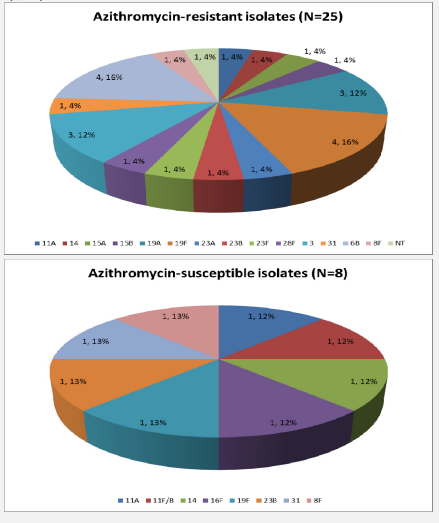
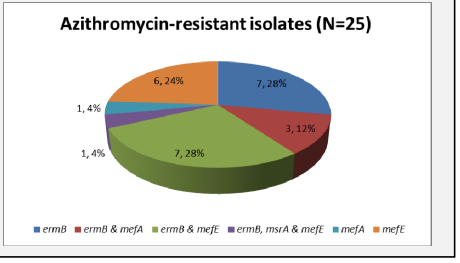


Figure 2. Resistance mechanisms found in the macrolide-resistant *S. pneumoniae* investigated (n, %)



Conclusions

- Solithromycin, unlike older macrolides, is mostly bactericidal for pneumococcus.
- Solithromycin exhibited superior MIC and MBC as compared with azithromycin.
- Importantly, MBC/MIC ratios for solithromycin were favourable.
- Ratio of MBC/MIC did not appear to relate to solithromycin MIC, serotype or genotype.
- These data support the use of solithromycin to treat pneumococcal infections, including those caused by macrolide-resistant strains.

References

1. Hackel M, Lascols C, Bouchillon S, Hilton B, Morgenstern D, Purdy J. Serotype prevalence and antibiotic resistance in *Streptococcus pneumoniae* clinical isolates among global populations. 2013. Vaccine 31; 4881-4887.
2. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob Agents Chemother. 40: 2562-6.
3. Clinical and Laboratory Standards Institute. 2012. Methods for Dilution Antimicrobial Susceptibility test for Bacteria That Grow Aerobically; Approved Standard-Eighth Edition. M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA, USA.
4. Clinical and Laboratory Standards Institute. 2014. Performance Standards for Antimicrobial Susceptibility testing; twenty-Fourth Informational Supplement. M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA, USA.
5. Clinical and Laboratory Standards Institute. 1999. Methods for determining bactericidal activity of antimicrobial agents; Approved Guideline. M26-A. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

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