In vitro Activity of Solithromycin (CEM-101) Against Macrolide-resistant *Streptococcus agalactiae* (Group B *Streptococcus*)

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Abstract (Amended)

Introduction: *Streptococcus agalactiae* (Group B *Streptococcus* or GBS), now recognized as a qualified infectious disease pathogen (QIDP) by the FDA, remains the leading cause of morbidity and mortality among infants in the United States. Although the incidence of GBS in pregnancy has decreased, penicillin and ampicillin minimal inhibitory concentrations (MICs) for GBS have risen, requiring higher doses for maternal and intrapartum treatment. In addition, co-infections with *Ureaplasma* remain untreated with penicillin. We have determined the *in vitro* activity of solithromycin (CEM-101) against 60 macrolide-resistant and 10 macrolide–susceptible GBS strains compared to that of other macrolides and penicillin.

Methods: Phenotypic characterization of macrolide-resistant strains was performed by double-disc diffusion testing. Multiplex PCR was used to identify the *ermB*, *ermTR*, and *mefA/E* genes from the GBS strains. Determination of MICs was carried out using the broth microdilution method according to CLSI guidelines. The Etest method was used for penicillin, azithromycin, clarithromycin and erythromycin as routinely tested in the laboratory.

Results: CEM-101 had a MIC_{50} of ≤0.008 μg/ml and a MIC_{90} of 0.015 μg/ml against macrolide-susceptible GBS. These MICs were lower than those displayed by penicillin (MIC_{50} of 0.032 and MIC_{90} of 0.047 μg/ml), the antibiotic agent of choice for prophylaxis and treatment of GBS infections. Against macrolide-resistant GBS, solithromycin had a MIC_{50} of 0.03 μg/ml and a MIC_{90} of 0.125 μg/ml. Against *ermB* strains, CEM-101 had a MIC_{50} of 0.03 μg/ml and a MIC_{90} of 0.06 μg/ml, while against *mefA* strains it had a MIC_{50} of 0.03 μg/ml and a MIC_{90} of 0.125 μg/ml. Against *ermB* strains, erythromycin, azithromycin, and clarithromycin MICs were mostly >256 μg/ml, while against mefA strains, erythromycin MIC_{50}s and MIC_{90}s were 6 and >256 mg/ml, azithromycin's MICs were 12 and >256 μg/ml and clarithromycin MIC_{50}s and MIC_{90}s were 1.5 and >256 μg/ml, respectively.

Conclusions: Overall, our results show that Solithromycin had lower or similar MICs compared to penicillin and good activity against macrolide-resistant GBS strains independent of their genotype or phenotype.

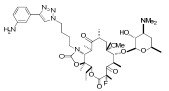
Introduction

Streptococcus agalactiae (group B streptococcus, GBS) is a common cause of severe infections in neonates, such as sepsis and meningitis. It is also an important pathogen causing bacteremia and endocarditis among elderly patients in immunocompromised subjects.[1] The highest GBS mortality and morbidity result from invasive infections in neonates, particularly in those with very low-birth weight.[1] Penicillin is the first-line antibiotic for treatment of GBS infection, as well as for intrapartum antibiotic prophylaxis to prevent early onset infection. Macrolides are the recommended second-line drugs and the first alternative in cases of beta-lactam allergy[1].

In 2008 GBS clinical isolates were identified with reduced penicillin susceptibility, in which an increase was observed in the MICs of beta-lactam antibiotics including penicillin (MICs of 0.25-1 mg/L)[1,8]. Also, the rates of erythromycin resistance have increased at different levels in various regions in the world[1, 10]. The need for new antibiotics active against GBS that could be used in infants as well as in pregnancy has been recognized by the placement of GBS on the proposed GAIN Qualified Infectious Diseases Pathogen (QIDP) list by the US FDA[2].

Solithromycin (CEM-101) is a fourth generation macrolide, a novel fluoroketolide, that is more potent in vitro than older macrolides[3,4]. The aim of this study was to evaluate the *in vitro* activity of solithromycin against a spectrum of *S.agalactiae* strains with different macrolide resistance genotypes and phenotypes.

Solithromycin (CEM-101)



Materials and Methods

Strains: 72 strains of *S. agalactiae*, from Brescia's main hospital (Spedali Civili) collected between 2005 and 2012, from urine (23), vagina (43), urethra (3) and rectal swab (3) samples. GBS strains were isolated by streak plating 1 to 10 ml of transport medium on ChromID streptoB agar plates (bioMérieux, St. Louis, MO, USA). The plates were incubated at 37° C for 18 to 24h in aerobic conditions. GBS was selected by the production of a pink pigment when grown aerobically on ChromID streptoB agar. GBS identification was performed by means of the VITEK^{tm} system (bioMérieux).

Antimicrobial resistance phenotype and genotype: Phenotypic characterization of macrolide resistant strains was performed by double-disc diffusion testing. Erythromycin (15 mg) and clindamycin (2 mg) discs were placed 20 mm apart. Isolates resistant to erythromycin with blunting of the clindamycin inhibition were of the $iMLS_B$ phenotype, isolates that demonstrated resistance to both erythromycin and clindamycin were of the $cMLS_B$ phenotypes, isolates showing resistance to erythromycin without blunting of the clindamycin inhibition zone were of the M phenotype, and isolates resistant to clindamycin yet susceptible or intermediate to erythromycin belonged to the L phenotype. Interpretative criteria were in according to CLSI guidelines[5]. A multiplex PCR was used to identify the *ermB*, *ermTR*, and *mefA/E* genes from the GBS strains and a separate PCR was used to amplify the *linB* gene[6].

Antimicrobial agents and MIC determination: Solithromycin was obtained from Cempra, Inc., Chapel Hill, NC. Determination of minimal inhibitory concentration (MIC) was carried out using the microdilution broth method according to CLSI guidelines[7]. In brief, an inoculum of approximately 5 x 10^5-5 x 10^6 CFU/ml was incubated with a concentration of solithromycin ranging from 0.008 to 4 μg/ml. *Streptococcus pneumoniae* ATCC 49619 was used as a quality control. Results were observed after 18 h of incubation at 37° C. For comparison to solithromycin (soli), penicillin (pen), azithromycin (azi), clarithromycin (clari) and erythromycin (ery) were used. The Etest method (Liofilchem, Italy) was used for all these antibiotics. Breakpoint interpretation was done according to EUCAST guidelines[8] and it was as follows: penicillin, ≤0.25 and >0.25 mg/L ; erythromycin, azithromycin and clarithromycin, ≤0.25 and >0.5 mg/L.

Statistical analysis: The $\chi 2$ test was used to evaluate the differences in distributions of isolates. A P value of < 0.05 was considered significant

Results

Table 1. The activities of solithromycin and the comparator antimicrobial agents against GBS are shown below

Organism	Antimicrobial drug	MIC (mg/L)		
		50%	90%	Range
Erythromycin-resistant GBS (n=62)	Solithromycin	0.03	0.125	≤0.008-1
	Penicillin	0.032	0.047	0.12-0.6
	Erythromycin[a]	>8	>8	0.5->8
	Erythromycin[b]	>256	>256	0.5->256
	Azithromycin	>256	>256	0.19->256
	Clarithromycin	>256	>256	0.25->256
Erythromycin-susceptible GBS (n=10)	Solithromycin	≤0.008	0.015	≤0.008-0.03
	Penicillin	0.032	0.047	0.012-0.047
	Erythromycin[a]	≤0.25	≤0.25	≤0.25
	Erythromycin[b]	0.047	0.047	0.012-0.064
	Azithromycin	≤0.125	≤0.125	0.019-0.19
	Clarithromycin	0.047	0.047	0.023-0.047

[a] *Broth microdilution test*
[b] *Etest*

Table 2. MICs (mg/L) of the different drugs against *Streptococcus agalactiae* (GBS) strains displaying $iMLS_B$ phenotype

Phenotype	Genotype	Soli	Pen	Azi	Clari	Ery[a]	Ery[b]
$iMLS_B$	*ermB*	0.06	0.047	6	1.5	2	4
$iMLS_B$	*ermB*	0.03	0.047	3	1	2	>8
$iMLS_B$	*ermTR*	≤0.008	0.023	6	1	2	1
$iMLS_B$	*ermB*	≤0.008	0.023	6	1.5	4	0.5
$iMLS_B$	*ermB*	≤0.008	0.032	2	0.75	1	0.5
$iMLS_B$	*ermTR*	≤0.008	0.047	8	0.75	3	0.5
$iMLS_B$	*ermTR+ mefA/E*	0.015	0.047	>256	8	12	>8

[a]*Etest*
[b]*Broth microdilution*

Table 3. MIC_{50} and MIC_{90} of solithromycin and comparator drugs against *S. agalactiae* strains with defined macrolide-resistant genotype

Drug	MIC (mg/L) for strains with different macrolide-resistant mechanism (no. of strains):			
	ermB (28)		mefA/E (22)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Solithromycin	0.03	0.06	0.03	0.125
Penicillin	0.032	0.047	0.047	0.047
Erythromycin[a]	>8	>8	>8	>8
Erythromycin[b]	>256	>256	6	>256
Azithromycin	>256	>256	12	>256
Clarithromycin	>256	>256	1.5	>256

[a]*Broth microdilution test*
[b]*Etest*

Table 4. MICs (mg/L) of the different drugs for *ermTR* gene and mixed resistance genotypes of *Streptococcus agalactiae*

Genotype	Soli	Pen	Azi	Clari	Ery[a]	Ery[b]
ermTR	0.03	0.032	>256	>256	>256	>8
ermTR	≤0.008	0.023	6	1	2	1
ermTR	≤0.008	0.047	8	0.75	3	0.5
erm TR + ermB	0.015	0.023	>256	1	4	1
erm TR + ermB	0.03	0.047	>256	>256	>256	>8
erm TR + ermB	0.03	0.047	>256	>256	>256	>8
erm TR + ermB	0.015	0.047	3	4	2	4
erm TR + ermB	0.03	0.047	>256	>256	>256	>8
ermB + mefA/E	0.06	0.012	>256	>256	>256	4
ermB + mefA/E	≤0.008	0.032	0.19	0.5	0.75	0.5
ermB + mefA/E	0.03	0.023	>256	>256	>256	>8
ermB + mefA/E	0.125	0.032	>256	>256	>256	>8
ermB+ ermTR+mefA/E	0.06	0.032	>256	>256	>256	>8
ermTR+mefA/E	0.015	0.047	>256	8	12	>8

[a]*Etest*
[b]*Broth microdilution test*

For solithromycin, most of the strains that displayed the $cMLS_B$ phenotype had a MIC between 0.03 and 0.06 mg/L, while for penicillin they had a range of MIC between 0.03 and 0.047 mg/L. Similar MIC distributions were observed for strains with the M phenotype. Most of the strains that had the $iMLS_B$ phenotype had a MIC of 0.047 mg/L for penicillin and a MIC of ≤0.008 mg/L for solithromycin.

All strains that had the $iMLS_B$ phenotype showed MICs of azithromycin exceptionally high compared to those of the other macrolides (Table 2). Similar unusual resistance has been noted in S. pyogenes[6].

Strains with the L phenotype had a MIC distribution between ≤0.008 and 0.015 mg/L for solithromycin and between 0.023 and 0.047 mg/L for penicillin. All the *ermB* gene carrying strains of *S. agalactiae* showed high resistance (MIC>256 mg/L) to clarithromycin and azithromycin. However, solithromycin showed a MIC_{90} of 0.06 mg/L against these strains (Table 3).

S. agalactiae strains carrying the *mefA/E* gene accounted for more than two-thirds of the macrolide resistant GBS isolates in this study. The MIC_{90} of solithromycin was 0.125 mg/L. MICs of *S. agalactiae* strains that had *ermA* (subclass *ermTR*) gene and the MICs of all the macrolide resistant strains that exhibited more than one resistance gene are shown in Table 4.

Evaluation of macrolide-resistant genotypes and phenotypes of GBS

Among the 62 macrolide-resistant clinical strains, 30 strains displayed the constitutive MLS_B phenotype, 21 the M phenotype, seven the inducible $iMLS_B$ phenotype, 4 the L phenotype. Three strains were L phenotypes and were erythromycin-intermediate and clindamycin-resistant strains and 1 L phenotype strain was an erythromycin-susceptible and clindamycin resistant strain by disk diffusion test. The *ermB* gene was present in 28 strains and it was mostly associated to the $cMLS_B$ phenotype, with a MIC of >256 mg/L for the older macrolides. The *mefA/E* gene was present in 22 strains, while *ermA* (subclass *ermTR)* was identified in 3 strains. The *linB* gene was not detected in any GBS strains and the L phenotypes observed were associated with the *ermB* gene (3 strains) and with the *mefA/E* gene (1 strain). Most of the strains carried a single resistance gene of either *ermB* or *mefA/E*. An exception occurred for 11 strains that exhibited a combination of *ermB* and *ermA* (subclass *ermTR*) (5 strains), of *ermB* and *mefA/E* genes (4 strains), of *ermA* (subclass *ermTR*) (1 strain), and one strain had all three macrolide resistance genes. An additional five strains with a susceptible phenotype possessed resistance genes including the *ermB* gene (2 strains), the *ermA* (subclass *ermTR*) (2 strains), and one strain that had both the *ermB* and *ermA* (subclass *ermTR*).

Discussion and Conclusions

The recent emergence of *S. agalactiae* strains with reduced penicillin susceptibility in Japan and the USA constitutes a problem for the use of this drug in prophylaxis[1,9]. The molecular analysis of these particular strains showed a mutagenic pathway comparable to that observed when the first beta-lactam resistant *S. pneumoniae* strains were isolated[9]. The emergence of a physiologically GBS pbp2x (Q557E) mutant is worrying, because the accumulation of additional mutations might lead to a complete penicillin resistance. This suggests a potential risk of therapeutic failure of intrapartum prophylaxis in the near future.

Traditionally the macrolides, and in particular erythromycin, have been considered the second-line choice of antibiotic in patients allergic to beta-lactams. However, resistance to macrolides and lincosamides has risen during the last decades with rates as high as 38%-41.9% in the United States[1]

The novel fluoroketolide solithromycin tested in this study demonstrated superior potency over older macrolides against all macrolide-resistant strains with MIC_{90} of 0.125 mg/L. These lower MICs suggest that this drug may be useful in the treatment of infections caused by these pathogens.

References

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