

J Deane¹, C Opiela¹, D Shah¹, D Sahn¹, K Keedy², A Sheets², P Fernandes²
¹Eurofins, Chantilly, VA, USA ²Cempra Inc., Chapel Hill, NC

Abstract (Amended)

Objectives: Solithromycin (SOL) is a novel fluoroketolide in late clinical development that has an *in vitro* activity spectrum different, and frequently more potent, than currently available macrolides. Against both *Enterococcus faecalis* (EFA) and *Enterococcus faecium* (EFM), including vancomycin-resistant strains, SOL has a MIC₅₀ of 2 mg/L. To further investigate the *in vitro* potency of SOL against enterococci, SOL's bactericidal activity was analyzed by time-kill kinetic analysis relative to that of linezolid (LZD).

Methods: Two strains each of vancomycin-susceptible (VS) and vancomycin-resistant (VR) EFA and EFM were analyzed. Broth microdilution testing according to CLSI M7 guidelines was used to obtain the drugs' MICs for each strain. Each strain was challenged with each drug at 2X, 4X, and 8X the MIC with CFU/mL determined at 0, 2, 4 and 24 hours. Bactericidal activity was defined as a 3 log₁₀ decrease in CFU/mL after 24 hours incubation relative to the concentration of the starting inoculum.

Results: The following table provides the log₁₀ CFU/mL reductions of initial inocula after 24 hours¹ at 2X, 4X, and 8X the SOL and LZD MICs.

Organism	MIC (mg/L)		2X MIC		4X MIC		8X MIC	
	SOL	LZD	SOL	LZD	SOL	LZD	SOL	LZD
EFA VR	0.25	1	0.81	0.68	1.13	1.3	1.06	1.25
EFA VS	0.06	2	-0.45	-0.76	0.05	0.02	0.14	0.18
EFM VR	0.06	2	0.81	0.23	1.83	0.58	1.83	1.13
EFM VS	0.06	2	2.92	1.71	2.76	1.21	2.92	1.71

¹ Negative numbers indicate growth greater than the initial inoculum

Conclusions: Although SOL MICs for each enterococcal strain tested were relatively low (0.06 - 0.25 mg/L), bactericidal activity was not achieved against any of the strains. As a comparator, LZD also failed to demonstrate bactericidal activity against any of the four strains tested. However, against the EFM-VS strain, SOL activity that approached cidalty was noted. This finding may warrant further analysis of a greater variety of enterococcal strains and indicates that SOL's spectrum can be quite different from those of older macrolides and ketolides.

Introduction

SOL is a novel fluoroketolide in late clinical development that has an *in vitro* activity spectrum different, and frequently more potent, than currently available macrolides. SOL has been reported to have activity against enterococci, with MIC values ≤2 mg/L against both EFA and EFM, including VR strains (Putnam 2011). To further investigate the *in vitro* potency of SOL against enterococci, SOL's bactericidal activity was analyzed by time-kill kinetic analysis relative to that of LZD.

Methods

- A VR and VS strain of EFA and of EFM were analyzed.
- The SOL and LZD MICs were established for each strain following CLSI M7 and M100 guidelines and the appropriate Eurofins SOPs. These MIC results were used for selection of the concentration of each drug that was used in the time-kill assays.
- The time-kill assay was done in accordance with Eurofins SOPs and the CLSI guidance document M26-A.
 - 5 x 10⁵ - 5 x 10⁸ CFU/mL log phased bacteria were inoculated in the presence of the antibiotic at 2X, 4X, and 8X the organisms' MIC as determined by broth microdilution testing.
 - A growth control, containing no antibiotic, was included for each strain tested.
 - At 0, 2, 4, and 24 hours post-inoculation, viable bacteria were quantified by serial dilution plating.
 - Bactericidal activity was defined as a ≥ 3-log₁₀ kill relative to the initial inoculum size.

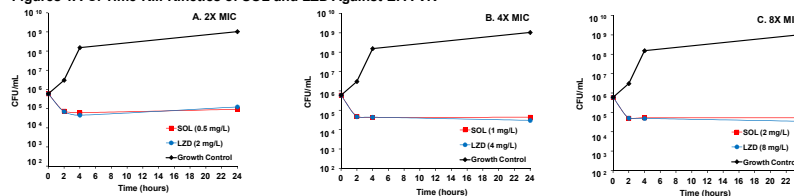
Results

Table 1. Log₁₀ CFU/mL Reduction After 24 Hours¹

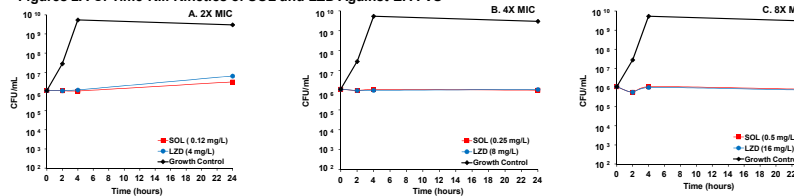
Organism	MIC (mg/L)		2X MIC		4X MIC		8X MIC	
	SOL	LZD	SOL	LZD	SOL	LZD	SOL	LZD
EFA VR	0.25	1	0.81	0.68	1.13	1.3	1.06	1.25
EFA VS	0.06	2	-0.45	-0.76	0.05	0.02	0.14	0.18
EFM VR	0.06	2	0.81	0.23	1.83	0.58	1.83	1.13
EFM VS	0.06	2	2.92	1.71	2.76	1.21	2.92	1.71

¹ Negative numbers indicate growth greater than the initial inoculum

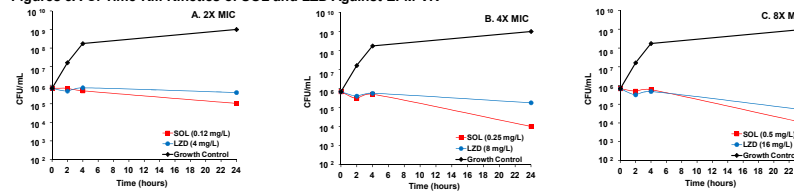
Figures 1A-C. Time-Kill Kinetics of SOL and LZD Against EFA-VR



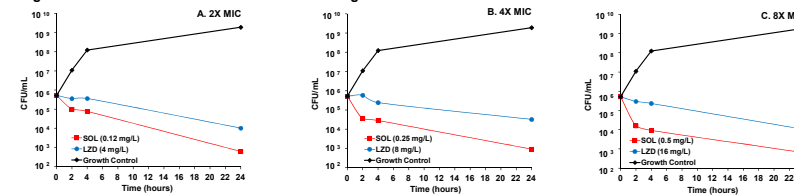
Figures 2A-C. Time-Kill Kinetics of SOL and LZD Against EFA-VS



Figures 3A-C. Time-Kill Kinetics of SOL and LZD Against EFM-VR



Figures 4A-C. Time-Kill Kinetics of SOL and LZD Against EFM-VS



Results

Results Summary:

- SOL was 4 to 32-fold more potent than LZD against the EFA and EFM strains evaluated.
- Against the EFA-VR strain, neither SOL nor LZD achieved a cidal level of activity after 24 hours, regardless of the multiple of the MIC tested (Figure 1A - C).
- Similarly, against the EFA-VS strain, neither SOL nor LZD achieved a cidal level of activity after 24 hours, regardless of the multiple of the MIC tested (Figure 2A - C).
- For the EFM-VR strain, SOL demonstrated some level of cidal activity at 4X and 8X the MIC, but did not achieve the required 99.9% drop in CFU. LZD did not exhibit cidal activity against this strain at any of the three concentrations tested (Figure 3A - C).
- Interestingly, for the EFM-VS strain, SOL closely approached or met the strict definition of cidalty with each multiple of the MIC tested. This level of cidalty was not observed with LZD (Figure 4A - C).

Summary and Conclusions

Few, if any, single drugs are bactericidal against EFA or EFM strains. Although the MICs for the enterococcal strains showed SOL was more potent than LZD, neither SOL nor LZD demonstrated bactericidal activity at any of the MIC multiples tested barring one notable exception (EFM-VS).

For the EFM-VS strain, each multiple of the SOL MIC closely approached the strict definition of cidalty.

Given that enterococci are refractory to the bactericidal activity of most single agents, and that macrolides are not typically considered bactericidal, this finding was unexpected.

This may be a strain-specific phenomenon, but further investigation into this finding is warranted.

References

- CLSI 2012. CLSI document M7-A9.
- CLSI 2012. CLSI document M100-S22.
- NCCLS (CLSI) 1999. CLSI document M26-A
- Putnam, S. D., H. S. Sader, D. J. Farrell, D. J. Biedenbach, and M. Castanheira. 2011. Antimicrobial characterisation of solithromycin (CEM-101), a novel fluoroketolide: activity against staphylococci and enterococci. *Int. J. Antimicrob. Agents* 37:39-45.

