D-591

Disk Zone and Minimal Inhibitory Concentration (MIC) Correlations for Testing Solithromycin (SOL), a Novel Fluoroketolide with Potent, Broad-Spectrum Activity

Contact info:
Dr. Daniel Sahm
Eurofins Medinet
dansahm@eurofins.com

J Deane[1], C Opiela[1], D Shah[1], A Sheets[2], K Keedy[2], P Fernandes[2], D F Sahm[1]

[1]*Eurofins, Chantilly, VA, USA,* [2]Cempra Pharmaceuticals, Chapel Hill, NC, USA

Abstract (Amended)

Background: SOL is a novel fluoroketolide that has an *in vitro* activity spectrum different and frequently more potent than currently available macrolides. As SOL is in late stage clinical development, this study was done to evaluate the relationship between broth MICs and disk zone diameter.
Methods: Isolates were concurrently tested against SOL by broth microdilution and disk diffusion (15 µg) in accordance with the appropriate CLSI guidelines. The organisms tested included *Staphylococcus* spp. (ST, [n=100]), *Enterococcus faecalis* (EF, [n=50]), *Streptococcus pneumoniae* (SP, [n=100]), beta-haemolytic streptococci (BH, [n=150]), *Haemophilus influenzae* (HI, [n=101]), and *Moraxella catarrhalis* (MC, [n=101]).
Results: As summarized in the table below for ST, EF, MC, and HI, a broad range of MICs and zone sizes were obtained that resulted in reasonable R^2 values. For SP and BH the activity of SOL was such that all MICs were ≤ 0.25 µg/mL. This lack of strains with higher MICs resulted in relatively lower R^2 values.

Organism	N	MIC Range (µg/mL)	Zone Range (mm)	R^2 Value
ST	100	0.015 - >32	6 - 39	0.80
EF	50	0.008 - 4	16 - 32	0.73
SP	100	0.002 - 0.25	21 - 35	0.25
BH	150	0.002 - 0.5	8 - 38	0.25
MC	101	0.002 - >32	6 - 47	0.47
HI	101	0.03 - >32	6 - 32	0.50

Conclusions: This study indicates that disk diffusion will be a reliable method for assessing the *in vitro* activity of SOL against the target bacterial species. The eventual establishment of disk interpretive breakpoints will need to await correlative MIC disk data from clinical trials and perhaps profiling a greater collection of isolates in an expanded disk/broth correlation study.

Introduction

SOL is a novel fluoroketolide that has an *in vitro* activity spectrum different and frequently more potent than currently available macrolides. As part of development, it is important to establish the feasibility and reliability of susceptibility testing by disk diffusion at a given disk mass. This study evaluated the correlation of broth microdilution MICs to disk zones when testing SOL by disk diffusion using a 15 µg disk mass as determined in a previous study. This study was conducted in accordance with CLSI M23 guidelines.

Methods

Strains.

- The 502 isolates consisted of non-duplicate, non-consecutive, clinically significant organisms from the Eurofins Repository.
- The organisms tested included: 100 ST, 50 EF, 100 SP, 150 BH (50 *S. agalactiae,* 50 *S. pyogenes,* 50 Group C, F, and G), 101 MC, and 101 HI.

Antimicrobial agents.

- SOL disks (15 µg) were commercially produced by BioRad (lot # 0L0010) and, for BH only, MAST (lot # 306628).
- Frozen broth microdilution panels were obtained commercially through ThermoFisher Scientific (Cleveland, OH, USA).
- Against ST, EC, HI, and MC, SOL was tested from 0.0005 µg/mL - 32 µg/mL. Against streptococci, SOL was tested from 0.0005 µg/mL - 4 µg/mL.

Testing Protocol.

- Each isolate was concurrently tested, using a common inoculum, for susceptibility to SOL by broth microdilution and disk diffusion in accordance with current CLSI guidelines (CLSI 2012).
- To obtain correlation results (R values), a linear regression analysis by the least-squares was performed (Pearson's correlation coefficient; MS Excel software) by plotting zone diameters against their respective MICs. Both on-scale, and off-scale broth microdilution results were included in the analysis.

Results

Table 1. Summary of Broth and Disk Correlation of SOL Against Target Gram-Positive and Gram-Negative Pathogens

Organism	N	MIC Range (µg/mL)	Zone Range (mm)	R^2 Value
ST	100	0.015 - >32	6-39	0.80
EF	50	0.008 - 4	16 - 32	0.73
SP	100	0.002 - 0.25	21 - 35	0.25
BH	150	0.002 - 0.5	8-38	0.26
MC	101	0.002 - >32	6-47	0.47
HI	101	0.03 - >32	6-32	0.50

Results (Continued)

Figure 1. SOL Broth and Disk Correlation Against ST (n = 100)

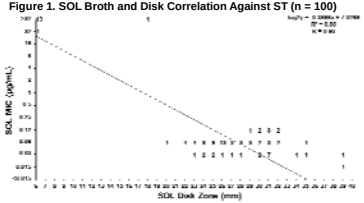


Figure 2. SOL Broth and Disk Correlation Against EF (n = 50)

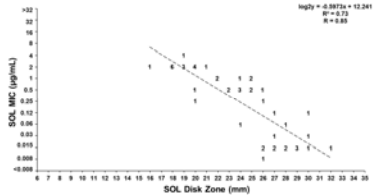


Figure 3. SOL Broth and Disk Correlation Against SP (n = 100)

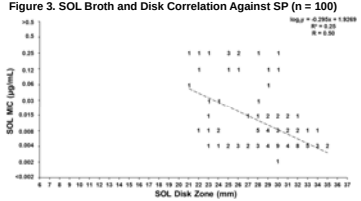


Figure 4. SOL Broth and Disk Correlation Against BH (n = 150)

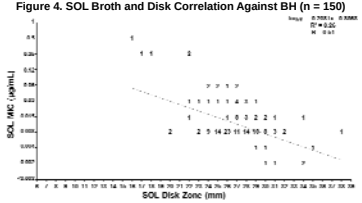


Figure 5. SOL Broth and Disk Correlation Against MC (n = 101)

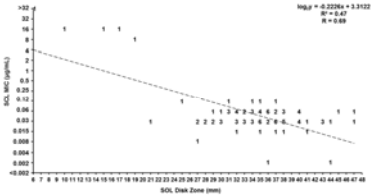
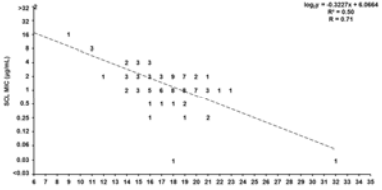


Figure 6. SOL Broth and Disk Correlation Against HI (n = 101)



A summary of the disk versus broth analysis for SOL against each organism group analyzed is provided in **Table 1**.

- For ST, EF, MC, and HI, a broad range of MICs and zone sizes were obtained that resulted in reasonable R^2 values.
- For SP and BH, the activity of SOL was such that all MICs were ≤ 0.25 µg/mL for SP and ≤ 0.5 µg/mL for BH. This lack of strains with higher MICs resulted in relatively lower R^2 values.

Results (Continued)

Figure 1. Disk versus Broth Results for ST

- All isolates tested had a SOL MIC range of 0.015 - >32 µg/mL.
- Basically two populations were encountered; those with SOL MICs ≥ 32 µg/mL and those with MICs ≤ 0.12 µg/mL.
- All isolates (except for one outlier) with MICs ≥ 32 µg/mL had a disk diffusion zone of 6 mm. The outlier had a MIC = 32 µg/mL and a zone of 20 mm. The identification of the isolate was confirmed, and on re-testing, the results were >32 µg/mL, 18 mm.
- All isolates with MICs ≤ 0.12 µg/mL gave zones in a range of 20 - 39 mm, with the majority of zones being between 22 and 31 mm.

Figure 2. Disk versus Broth Results for EF

- All isolates tested gave a SOL MIC range of 0.008 - 4 µg/mL and regression analysis showed a good correlation (R^2 = 0.73) with decreasing zones correlating with increasing MICs.

Figure 3. Disk versus Broth Results for SP

- The majority (70%) of isolates had SOL MICs of ≤ 0.008 µg/mL and no "resistant" isolates were tested using a tentative resistant breakpoint of > 1 µg/mL for SP.
- It appears that the disk test will be effective for SP, but unless strains with higher MICs are encountered and tested, setting intermediate and resistant breakpoints will not be possible. In such cases only a susceptible category may be established.

Figure 4. Disk versus Broth Results for BH

- The organisms tested were comprised of *S. agalactiae*, *S. pyogenes*, and Group C, F, and G BH
- No isolates had a SOL MIC above 0.5 µg/mL or a zone diameter < 16 mm.
- The majority of strains had MICs between 0.008 and 0.015 µg/mL and gave zones between 24 and 30 mm.
- It appears that the disk test will be effective for testing BH, but unless strains with higher MICs are encountered and tested, ultimately setting intermediate and resistant breakpoints will be difficult. In absence of such strains only a susceptible category would be possible.

Figure 5. Disk versus Broth Results for MC

- The majority of strains (87%) had SOL MICs between 0.03 and 0.12 µg/mL with a corresponding zone range from 27 to 39 mm.
- Five isolates had a SOL MIC between 8 and >32 µg/mL and zones between 6 and 19 mm. SOL MICs >0.5 µg/mL have not previously been reported for MC; however, the MIC-zone correlation in these few isolates seems reasonable (Farrell, DJ et al. 2009, Farrell, DJ et al. 2010, Biedenbach, DJ et al 2012).
- It appears that the disk test will be effective for MC, but unless more strains with higher MICs are encountered and tested setting intermediate and resistant breakpoints will not be possible. In such cases only a susceptible category may be established (as was mentioned for streptococci).

Figure 6. Disk versus Broth Results for HI

- The majority of isolates (92%) had SOL MICs between 0.25 and 4 µg/mL with zones of inhibition that ranged from 12 to 23 mm.
- Six out of 101 isolates had SOL MICs from 8 to >32 µg/mL with zones that ranged from 6 to 11 mm.
- It appears that the disk test will be effective for HI.

Conclusions

Results of this study indicate that disk diffusion will be a reliable method for assessing the *in vitro* activity of SOL against the target bacterial species. The eventual establishment of disk interpretive breakpoints will need to await correlative MIC and disk data from clinical trials, and perhaps profiling a greater collection of isolates in an expanded disk/broth correlation study.

References

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