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Comparison of Solithromycin MICs Generated by BSAC and CLSI Methods

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Introduction and Purpose

Solithromycin (CEM-101) is a fourth generation macrolide, the first fluoroketolide that has completed two global Phase 3 trials in Community-Acquired Bacterial Pneumonia (CABP) where it has been effective and well tolerated. Solithromycin has been shown to have activity against macrolide–resistant strains. Regulatory approval is being sought in the EU and the US for use in treating moderate to moderately severe CABP. Therefore, it is important that susceptibility testing methods used in European Countries and in the US are in agreement.

The purpose of this study was to determine the comparability of minimum inhibitory concentrations (MICs) of CEM-101 (solithromycin) generated by dilution methods for susceptibility testing as recommended by Clinical and Laboratory Standards Institute (CLSI) and The British Society for Antimicrobial Chemotherapy (BSAC).

Methods

Drugs. MICs of CEM-101 (Cempra, Lot# EKS11646), were determined. Drug was dissolved and diluted for testing per recommendations in CLSI M100-S25[1].

Organisms. Thirty isolates each of *Streptococcus pneumoniae* (15 erythromycin-susceptible and 15 erythromycin-resistant), *Haemophilus influenzae*, and *Staphylococcus aureus* (15 erythromycin-susceptible and 15 erythromycin-resistant), and 15 isolates each of *Moraxella catarrhalis* and *Enterobacteriaceae* cultured from specimens of patients submitted to the Clinical Microbiology Laboratories at the University of Rochester Medical Center, Rochester, NY, in 2014 were included in this study; study isolates were selected to represent a range of CEM-101 (solithromycin) MICs. Organisms were biochemically identified according to standard clinical microbiological techniques and stored frozen at -80˚ C until tested; erythromycin susceptibility for indicated strains was determined by agar disk diffusion testing as described by CLSI M2-A12[2] and CLSI M100-S25 or Vitek 2 semi-automated susceptibility test method (bioMerieux, Inc., Durham, NC).

MIC Determinations. Prior to testing, *S. pneumoniae* was subcultured on Tryptic Soy Agar with 5% sheep blood (Becton Dickinson, Sparks, MD) and incubated in ambient air with 5% CO_2 at 35-37˚ C for 18-20 hours. Prior to testing, *S. aureus*, *M. catarrhalis* and *Enterobacteriaceae* were subcultured on Tryptic Soy Agar with 5% sheep blood (Becton Dickinson, Sparks, MD) and incubated in ambient air at 35-37˚ C for 18-20 hours. Prior to testing, *H. influenzae* was subcultured on Chocolate Agar II (Becton Dickinson, Sparks, MD) and incubated in ambient air with 5% CO_2 at 35-37˚ C for 18-20 hours.

MICs of CEM-101 (solithromycin) for all isolates were determined by agar dilution methodology and broth microdilution methodology as described by CLSI M7-A10[3]. In addition, MICs of CEM-101 (solithromycin) were also determined by agar dilution methodology and broth microdilution as described by BSAC (J.M. Andrews et al[4]). Mueller-Hinton broth (Difco, Lot# 3353060), Mueller-Hinton agar (Difco, Lot# 3016087), Iso-Sensitest broth (Oxoid, Lot# 1484803) and Iso-Sensitest Agar (Oxoid, Lot# 1483970) were used in this study.

MIC endpoints for drugs were read as the concentrations at which no growth was observed by visual inspection after incubation. The performance of tests reagent (including drug potency) and equipment, and test personnel was monitored using *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247 as recommended by CLSI M100-S25. MICs for appropriate quality control organisms tested in parallel with clinical isolates were within acceptable ranges as recommended by CLSI.

Results

Comparisons of MICs of CEM-101 (solithromycin) determined by the different methods were made by analyzing scatterplots and by calculating the doubling dilution differences (DD) between MICs. When doubling dilution differences between MICs were compared, good correlation was observed for *H. influenzae* and *M. catarrhalis* with the BSAC agar dilution method compared to the CLSI broth microdilution method (see **Table 1 and Figures 1-4**). For all species and regardless of the MIC method comparison, 97% to 100% of CEM-101 (solithromycin) MICs were within ±2DD for *S. aureus* and *S. pneumoniae*, and 90% to 100% of MICs were within ±2DD for *M. catarrhalis* and *H. influenzae*. It is noted, that in general solithromycin was less active in agar than in broth medium which is likely noted in the pathogens with the lower MICs.

For most comparisons, good to excellent correlations between MICs determined by the different methods were observed in scatterplot comparisons (see **Table 2**). For *S. aureus* and *M. catarrhalis*, regression analysis demonstrated an almost linear relationship between the results by the different methods with R^2 and correlation coefficients ranging from 0.95 to 0.99. The relationship between results by different methods for *S. pneumoniae* and *H. influenzae* was good but less linear as demonstrated by R^2 and correlation coefficients ranging from 0.7 to 0.9.

Table 1: Doubling Dilution Comparisons for BSAC vs CLSI Methods

	BSAC Agar vs CLSI Broth		BSAC Agar vs CLSI Agar		CLSI Broth vs CLSI Agar		BSAC Broth vs BSAC Agar		BSAC Broth vs CLSI Broth	
Organism	±1DD	±2DD	±1DD	±2DD	±1DD	±2DD	±1DD	±2DD	±1DD	±2DD
H. influenzae	87%	97%	90%	100%	100%	100%	67%	90%	87%	100%
M. catarrhalis	80%	100%	100%	100%	47%	93%	20%	100%	80%	100%
S. aureus	77%	97%	90%	100%	83%	100%	70%	100%	83%	100%
S. pneumoniae	73%	97%	100%	100%	87%	97%	97%	100%	83%	100%

Table 2: Correlation of MICs Generated by Different Methods as Determined by Scatterplot

	CLSI Broth vs BSAC Agar		CLSI Agar vs BSAC agar		CLSI Agar vs CLSI Broth		BSAC Agar vs BSAC Broth		CLSI Broth vs BSAC Broth	
Organism	R^2	Pearson Coefficient	R^2	Pearson Coefficient	R^2	Pearson Coefficient	R^2	Pearson Coefficient	R^2	Pearson Coefficient
M. catarrhalis	0.98	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.99
S. aureus	0.95	0.98	0.98	0.99	0.96	0.98	0.95	0.98	0.97	0.99
S. pneumoniae	0.75	0.87	0.91	0.95	0.79	0.89	0.84	0.92	0.86	0.93
	HTMB vs BSAC Agar		**HTMA vs BSAC Agar**		**HTMA vs HTMB**		**BSAC Agar vs BSAC Broth**		**HTMB vs BSAC Broth**	
Organism	R^2	Pearson Coefficient	R^2	Pearson Coefficient	R^2	Pearson Coefficient	R^2	Pearson Coefficient	R^2	Pearson Coefficient
H. influenzae	0.72	0.85	0.82	0.91	0.85	0.92	0.53	0.73	0.74	0.86

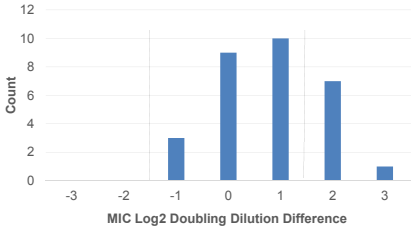

Figure 1: *S. pneumoniae* BSAC Agar vs CLSI Broth

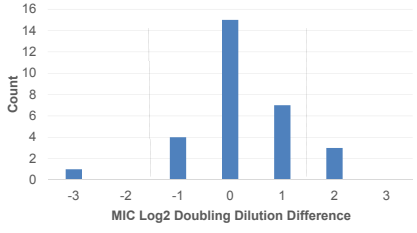

Figure 2: *H. influenzae* BSAC Agar vs HTMB

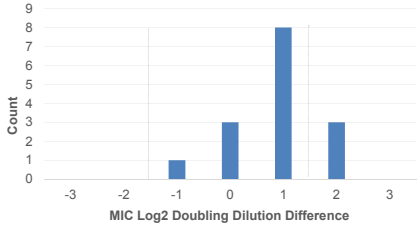

Figure 3: *M. catarrhalis* BSAC Agar vs CLSI Broth

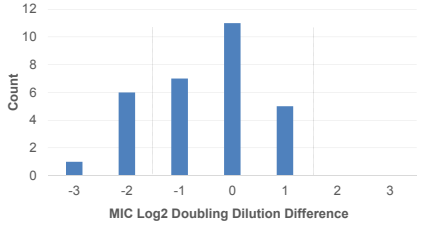

Figure 4: *S. aureus* BSAC Agar vs CLSI Broth

Note: Negative numbers indicate BSAC Agar MICs were lower than those obtained with CLSI Broth.
Positive numbers indicate BSAC Agar MICs were higher than those obtained with CLSI Broth.

Conclusions

There is good agreement between MICs of CEM-101 (solithromycin) determined by CLSI and BSAC agar dilution methods and good agreement between methods when MICs are determined by broth dilution methods. When comparing MICs of solithromycin determined by BSAC agar dilution method to those determined by CLSI broth dilution, it was observed that although most MICs for *S. aureus* and *S. pneumoniae* are within 1 doubling dilution, there is a tendency toward lower MICs for *S. aureus* and higher MICs for *S. pneumoniae* when testing by the BSAC agar dilution method. We have noted that solithromycin could be 1-2 doubling dilution less active in agar than in broth medium and this could be related to solithromycin binding to the solid agar matrix.

References

1. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100-S25; **2.** Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – Twelfth Edition. CLSI document M2-A12; **3.** Clinical and Laboratory Standards Institute. Methods for Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Tenth Edition. CLSI document M7-A10; **4.** Andrews, J.M. 2006. BSAC Standardized Disk Susceptibility Testing Method (version 5). J. Antimicrob. Chemother. **58:**511-529.

Disclosures

Kara Keedy, Prabhavathi Fernandes, and Jie Li are employees of Cempra, Inc. D. Hardy and D. Vicino conducted the work under contract to Cempra, Inc.