F1-3984

Assessment of CEM-101 Susceptibility Testing Conditions and Optimization of Disk Diffusion Methods

RN JONES, TR FRITSCHE, HS SADER, DJ BIEDENBACH, PR RHOMBERG
JMI Laboratories, North Liberty, IA

ICAAC/IDSA 2008
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

ABSTRACT

Background: CEM-101, a novel macrolide-ketolide, has potent activity against susceptible (S) and resistant (R) respiratory tract infection pathogens. To prepare it for clinical trials, in vitro S testing details for MIC methods and the selection of disk diffusion (DD) CEM-101 content were established.

Methods: CLSI broth microdilution (BMD) was used and the following test conditions were modified to determine effects on CEM-101 activity; anaerobic and CO_2 atmosphere; 5 x 10^3 and 5 x 10^7 inoculum; LHB and HTM; pH 5, 6 and 8; human serum protein at 5, 10 and 20%; calcium at 3.7 and 50 mg/L and use of polysorbate-80 (P-80) surfactant. Simultaneous changes in the pH and protein were also tested. CEM-101 DD tests with 2-, 5-, 10-, 15- and 30-µg versus 70 selected S and R strains were assessed.

Results: By changing BMD test conditions, only the following resulted in significantly (≥4-fold) elevated CEM-101 MIC results: high inoculum (5 x 10^7), P-80 at 2% (see Figure 1) and pH 5 or 6. pH effect was muted for pH 6 by presence of 10% human serum protein. Scattergrams with CEM-101 MIC values and zone diameters produced r values of 0.93-0.97 and the 15-µg disk (like other macrolides) provided best discrimination of S and R strains of staphylococci, enterococci and *H. influenzae.*

Conclusions: CEM-101 S testing by CLSI methods appears to be optimized for clinical trials using published BMD procedures without P-80. The 15-µg CEM-101 DD test accurately assesses this new agent's activity.

INTRODUCTION

CEM-101 is a novel macrolide-ketolide agent to be administered orally with a spectrum of activity most similar to telithromycin. This agent has probable clinical applications against community-acquired respiratory tract infections (CA-RTI) and uncomplicated skin and skin structure infections (uSSSI) caused by *Staphylococcus aureus*, *Streptococcus* spp., *Enterococcus* spp. and fastidious Gram-negative species, such as *Haemophilus influenzae* and *Moraxella catarrhalis.*

To prepare for CEM-101 testing during clinical trials, the Clinical and Laboratory Standard Institute (CLSI; formerly the NCCLS) reference in vitro broth microdilution (BMD) susceptibility method with varying testing parameters were performed (modifications in the incubation atmosphere, inoculum concentration, growth medium, pH, calcium ion content, serum protein concentration, and a surfactant effect). A disk content ranging study was performed to optimize the discrimination of susceptible and resistant isolate populations. Using the 15-µg content disk, a MIC/disk diffusion (DD) scattergram experiment was performed.

MATERIALS AND METHODS

Broth microdilution (BMD) susceptibility testing:

The CLSI BMD method (M7-A7, 2006) was performed for susceptibility testing of CEM-101 under standard conditions as well as the following alterations:

- Anaerobic and CO_2 incubation environment
- 5x10^3 and 5x10^7 inoculum concentrations
- Lysed horse blood (LHB) and Haemophilus Test Medium (HTM)
- Media pH at 5, 6 and 8
- 5, 10, and 20% added pooled human serum
- 3.7 and 50 mg/L calcium ion content
- Polysorbate-80 (P-80) supplementation

Disk content ranging study:

The CLSI standard DD method (M2-A9, 2006) was performed with investigator-produced 2-, 5-, 10-, 15-, and 30-µg CEM-101 disks to determine the optimum disk content to discriminate zone diameters of possible susceptible and resistant organism populations.

MIC/disk diffusion zone study:

A total of 50 isolates including staphylococci, enterococci, *H. influenzae*, and Enterobacteriaceae were selected to determine the correlation of the zone diameters produced with the 15-µg CEM-101 content disk plotted against BMD MIC results.

RESULTS

- MIC test results for selected bacterial isolates tested under standard CLSI susceptibility testing conditions and concurrently with modifications to incubation atmosphere, inoculum density, growth media, pH, serum proteins and calcium content are summarized in Table 1.
- The only modified testing conditions to significantly (≥4-fold MIC change) affect the CEM-101 results, compared to standard test conditions, were elevated inoculum concentration (5x10^7) for two strains of *S. aureus*, added human serum (20%) for one strain of *K. pneumoniae* and lowering the media pH to 5 or 6 for all strains. (Table 1).
- When testing the CEM-101 MIC with simultaneous changes to the medium pH and adding 10% human serum, produced a muting of the effect to increase CEM-101 MIC results at pH 6 but not for pH 5 (Table 2). Human serum enhanced the action of CEM-101 (Table 2), a reproducible phenomenon probably mediated by higher pH of pooled serum.
- Figure 1 shows the average of CEM-101 susceptibility results for five *S. aureus* strains tested in the presence of 10-fold decreasing levels of P-80 (2 – 0.000002%). Only the very high levels (0.2 and 2%) adversely affected the MIC results.
- Disks prepared with 2-, 5-, 10-, 15- and 30-µg of CEM-101 were tested against selected isolates representing staphylococcal, enterococcal, Enterobacteriaceae, *P. aeruginosa* and fastidious CA-RTI pathogens showed corresponding inhibition zones that increased 5 to 7 mm from the 2- to 30-µg disk (Data not shown). Best discrimination of the different levels of CEM-101 activity was observed for the 15-µg disk, a disk drug content commonly used for other drugs in this class.
- Figure 2 shows the excellent correlation (r=0.92) of the scattergram plot results when testing 50 isolates by BMD and DD (15-µg disk only). Three distinct populations were observed; highly susceptible – staphylococci & streptococci, moderately susceptible – *H. influenzae*; and resistant – enteric bacilli, non-fermentative bacilli and MLS_B-resistant staphylococci.

Table 1. CEM-101 reference broth microdilution MIC results when testing conditions are varied from the standardized procedures (CLSI M7-A7, 2006).

		Atmosphere		Inoculum (CFU/ml)		Media		pH			Serum (%)			Calcium (mg/L)	
Organism/strain no.	Standard conditions[a]	Ana	CO_2	5 x 10^3	5 x 10^7	LHB	HTM	5	6	8	5	10	20	3.7	50
S. aureus															
ATCC 25923	0.12	0.12	0.12	0.12	0.25	0.06	0.12	4[b]	1	0.12	0.12	0.12	0.12	0.12	0.06
ATCC 29213	0.12	0.12	0.12	0.12	0.5	0.06	0.12	4	1	0.12	0.12	0.12	0.12	0.12	0.06
120-1606A	0.12	0.25	0.25	0.12	0.5	0.06	0.25	8	2	0.12	0.12	0.12	0.12	0.12	0.12
CoNS[c]															
63-263A	0.25	0.5	0.5	0.12	0.5	0.12	0.25	16	4	0.25	0.25	0.25	0.12	0.25	0.25
75-2023A	0.12	0.25	0.12	0.12	0.25	0.06	0.12	0.5	2	0.12	0.12	0.12	0.12	0.12	0.06
Enterococci[d]															
ATCC 29212	0.06	0.12	0.06	0.06	0.12	0.03	0.06	0.25	0.25	0.03	0.06	0.06	0.06	0.06	0.03
106-765A	0.06	0.12	0.06	0.06	0.12	0.03	0.06	0.25	0.25	0.03	0.06	0.06	0.06	0.06	0.03
2-125A	0.12	0.12	0.12	0.06	0.25	0.03	0.06	1.0	0.5	0.06	0.12	0.12	0.12	0.06	0.06
E. coli															
ATCC 25922	8	16	16	8	16	8	16	>64	>64	8	8	16	4	8	16
K. pneumoniae															
117-24A	32	64	32	32	64	32	64	>64	>64	16	32	16	8	32	32
S. pneumoniae															
ATCC 49619	0.015	-	-	-	-	0.015	-	-	-	-	-	-	-	-	-
H. influenzae															
ATCC 49247	2	-	-	-	-	-	2	-	-	-	-	-	-	-	-

a. Ambient air, 5 x 10^5 CFU/ml inoculum, MHB, pH 7.2-7.4, no serum and calcium at 25 mg/L.
b. Underlined values show MIC variations of ≥four-fold.
c. CoNS = coagulase-negative staphylococci (*S. epidermidis* and *S. haemolyticus*).
d. Includes: *E. faecalis* (two) and *E. faecium* (one).

Table 2. In vitro MIC value influences of medium pH ± 10% human serum protein on CEM-101 activity tested against five *S. aureus* strains.

		CEM-101 MIC at pH (µg/ml):			
Organisms	10% serum	5.0	6.0	7.2-7.4	8.0
S. aureus 120-1606A	-	4	1	0.12[a]	0.12
	+	1	0.12	0.06	0.03
S. aureus 082-12293A	-	4	1	0.5[a]	0.25
	+	2	0.5	0.12	0.06
S. aureus 090-15105A	-	2	0.5	0.12[a]	0.12
	+	1	0.12	0.06	≤0.015
S. aureus ATCC 29213	-	4	0.5	0.12[a]	0.06
	+	1	0.12	0.06	0.06
S. aureus ATCC 25923	-	4	1	0.12[a]	0.12
	+	1	0.12	0.06	0.03

a. Reference MIC at pH 7.2-7.4 without added human serum proteins.

Figure 1. Average CEM-101 MIC values for five tested *S. aureus* strains when combined with various concentrations of a surfactant (polysorbate-80; P-80 at 0.000002 to 2%).

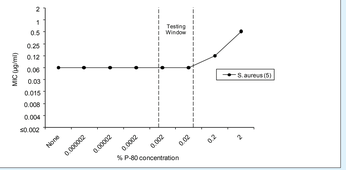
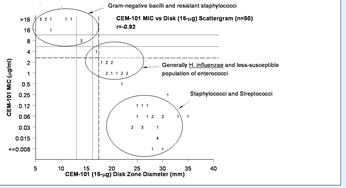


Figure 2. Proposed CEM-101 breakpoints to be applied to the clinical trials and further diagnostic test development (susceptible at >=17 mm [<=4 µg/ml] and resistant at <=13 mm [>=16 µg/ml]). Alternative susceptibility breakpoints are illustrated by the broken lines.



CONCLUSIONS

- CEM-101 MIC results were minimally influenced by variations from the CLSI BMD standard testing conditions.
- P-80 concentrations below 0.2% did not influence CEM-101 MIC results, however high levels ≥0.2% may be antagonistic to CEM-101 activity; P-80 use for CEM-101 BMD testing is not recommended.
- The use of a 15-µg CEM-101 disk was able to accurately discriminate highly resistant strains (MICs, ≥8 µg/ml) from those with susceptible CEM-101 MIC values (two levels of activity were noted).
- Excellent correlation between CLSI reference BMD MIC results and DD zone diameters using the 15-µg disk was observed, indicating acceptability of these reagents for use in CEM-101 clinical trial testing.

SELECTED REFERENCES

1. Clinical and Laboratory Standards Institute (2006). *M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition.* Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2006). *M2-A9, Performance standards for antimicrobial disk susceptibility tests; approved standard - ninth edition.* Wayne, PA: CLSI.
3. Clinical and Laboratory Standards Institute (2008). *M100-S18, Performance standards for antimicrobial susceptibility testing, 18th informational supplement.* Wayne, PA: CLSI.
4. Fuchs, PC, Barry, AL, Brown, SD (2001). Influence of variations in the test methods on susceptibility of *Haemphilus influenzae* to ampicillin, azithromycin, clarithromycin, and telithromycin. *J Clin Microbiol* 39: 43-46.
5. Jones, RN, Fritsche, TR, Ge, Y, Kaniga, K, Sader, HS (2005). Evaluation of PPI-0903M (T91825), a novel cephalosporin: Bactericidal activity, effects of modifying in vitro testing parameters and optimization of disc diffusion tests. *J Antimicrob Chemother* 56: 1047-1052.
6. National Committee for Clinical Laboratory Standards (2001). *M23-A2, Development of in vitro susceptibility testing criteria and quality control parameters; approved standard - second edition.* Wayne, PA: CLSI.