MIC Quality Control Guidelines and Disk Diffusion Test Optimization for CEM-101, a Novel Fluoroketolide

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The development of diagnostic susceptibility tests for CEM-101, a new fluoroketolide, was addressed by structured studies to determine the optimal disk diffusion test concentration and effects of various testing conditions or supplements and to establish the quality control (QC) ranges for reference broth microdilution tests. The 15-µg CEM-101 disk was selected, and MIC ranges for a total of four QC organisms were proposed, with only three doubling dilutions each that included 95.6 to 99.7% of values reported from the eight-laboratory investigation.

CEM-101 is an investigational fluoroketolide antimicrobial agent with spectrum features superior to existing macrolides and most similar to telithromycin (1, 6, 8–16). This agent has potential clinical applications against community-acquired bacterial pneumonia (CABP) and uncomplicated skin and skin structure infections (uSSSI) caused by Staphylococcus aureus, Streptococcus spp., some Enterococcus spp., and fastidious Gram-negative pathogens such as Haemophilus influenzae and Moraxella catarrhalis (12, 15, 16). To prepare for CEM-101 in vitro susceptibility testing during clinical trials, the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) standard broth microdilution susceptibility method (3) was performed with various testing parameters. A disk content ranging study was initiated to optimize the discrimination of susceptible and resistant isolate populations (2–5). Using the selected 15-µg CEM-101 disk concentration, a pilot MIC/disk diffusion zone diameter scattergram experiment was performed (4). In addition, a CEM-101 broth microdilution quality control (QC) study utilized the CLSI M23-A3 guideline (4) design. The QC study employed eight laboratories, three different manufacturers of media, and several antimicrobial control agents. The results are presented as proposed QC ranges in µg/ml for four ATCC strains (Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212, Streptococcus pneumoniae ATCC 49619, and Haemophilus influenzae ATCC 49247) (4, 5).

The CLSI M07-A8 method (3) was utilized for susceptibility testing of CEM-101 under standard conditions as well as with the following test alterations (12 organisms) to assess the impact of changing test conditions: anaerobic and CO2 incubation environment; concentrations of 5 x 103 and 5 x 107 CFU/ml inoculum; lysed horse blood (LHB) and Haemophilus test medium (HTM) broths; medium pHs at 5, 6, and 8; 5, 10, and 20% added pooled human serum; trace and 50-mg/liter calcium ion contents; and polysorbate 80 (P-80) supplementation (0.000002 to 2.0%). CLSI standardized disk diffusion method M02-A10 (2) was performed with investigator-prepared 2-, 5-, 10-, 15-, and 30-µg CEM-101 disks to determine the optimal disk content to discriminate zone diameters for the susceptible or wild-type population from the resistant organism population. A total of 50 additional isolates, including staphylococci, enterococci, H. influenzae, and Enterobacteriaceae, were selected to calculate the correlation of zone diameters produced with the optimized 15-µg CEM-101 disk content when plotted against reference MIC test results (2–5).

Eight laboratories were recruited to provide sufficient information for the QC investigation (4). Four cation-adjusted Mueller-Hinton broth (CAMHB) media included lots produced by Difco Laboratories (Detroit, MI), Becton Dickinson (BD, Sparks, MD), and Oxoid (Hampshire, United Kingdom). Four CAMHB lots supplemented with 2 to 5% lysed horse blood or HTM were also supplied by Difco, BD, and Oxoid. CEM-101 was provided by Cempra Pharmaceuticals, Inc. (Chapel Hill, NC), and azithromycin, erythromycin, and clarithromycin were acquired from Sigma-Aldrich (St. Louis, MO). Panels were prepared by a certified good manufacturing practice (GMP) laboratory source (Trek Diagnostics, Cleveland, OH).

Internal QC was established throughout all phases of these studies by using erythromycin tested against S. aureus and E. faecalis as a “peer drug” comparator agent and azithromycin and/or clarithromycin for S. pneumoniae and H. influenzae testing (4). Appropriate inoculum concentrations were monitored by performing colony counts with the inoculated broth microdilution trays, which were subcultured onto drug-free
agar plates. The average colony counts among the participating centers ranged from $2.6 \times 10^3$ to $5.7 \times 10^5$ CFU/ml, which is an acceptable performance (3–5).

The CEM-101 reference MIC results for 12 selected bacterial strains were tested under various conditions. Compared to standardized testing conditions (3), CEM-101 MICs were significantly increased (≥4-fold) only with elevated inoculum concentrations at $5 \times 10^7$ CFU/ml (two S. aureus strains) and when the medium pH was less than 7. An extended experiment of a combined effect of medium pH and 10% human serum protein on five S. aureus strains illustrated that the adverse influences of low medium pH on CEM-101 were moderated by human serum protein (data not shown). In fact, one Klebsiella pneumoniae strain was 4-fold more susceptible to CEM-101 when tested in CAMHB with pooled human serum.

Recent standardization of large-molecule lipoglycopeptides (dalbavancin, oritavancin, and telavancin) requires the use of 0.002 to 0.02% polysorbate 80, a surfactant, to minimize drug binding to plastic panels (3, 5). When CEM-101 was tested in the presence of 0.000002 to 0.02% polysorbate 80, no change in potency against five S. aureus strains was observed (Fig. 1). However, when the surfactant concentrations were increased to 0.2 or 2%, an antagonistic result was detected (the CEM-101 MIC increased to 1 μg/ml, a 16-fold elevation). Clearly, this new fluoroketolide will not require the adjunctive use of polysorbate 80.

An optimization of the disk diffusion test was performed in two phases, where CEM-101 disks were prepared at concentrations of 2, 5, 10, 15, and 30 μg, and organisms with CEM-101 MICs representing the indicated wild-type species (staphylococci, streptococci, enterococci, and H. influenzae) were tested and compared to generally resistant pathogens (10 total strains). The best discrimination of susceptible wild-type strains from resistant (MIC, ≥16 μg/ml) organisms was achieved with CEM-101 disks with a ≥10-μg content. The disk concentration routinely used for macrolides (azithromycin, clarithromycin, and erythromycin) and telithromycin (also a ketolide) has been 15 μg (5); therefore, this disk content was recommended for further development. A total of 50 strains were tested in duplicate (average zone and MIC results are presented in Fig. 2) to confirm initial findings. Figure 2 illustrates the positions of various CEM-101 zone diameter and MIC results ($r = 0.92$), leading to the potential zone diameter breakpoint for H. influenzae of ≥17 mm (MIC, ≤4 μg/ml), which is most similar to that of telithromycin or azithromycin. Larger zones and lower MIC breakpoints could be considered for the staphylococcus and streptococcus (including S. pneumoniae) isolates during clinical trial development (Fig. 2).

![FIG. 1. Average CEM-101 MICs for five tested S. aureus strains when combined with various concentrations of a surfactant (polysorbate 80 at 0.000002 to 2%).](image)

![FIG. 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 horizontal and vertical lines.](image)
The CEM-101 MIC QC trial followed the design dictated by the CLSI M23-A3 document (4) for the M07-A8 method (3). Table 1 shows the remarkable consistency of CEM-101 MICs generated for the four studied QC strains. All QC organisms produced clear modal and median MICs, and the following three log2 dilution MIC QC ranges were proposed for each organism: 0.015 to 0.06 μg/ml for E. faecalis ATCC 29212 (95.6% of results in the calculated range), 0.03 to 0.12 μg/ml for S. aureus ATCC 29213 (96.6%), and 0.004 to 0.015 μg/ml for S. pneumoniae ATCC 49619 (99.4%) (also see Fig. 3), and 1 to 4 μg/ml for H. influenzae ATCC 49247 (99.7%). All internal quality assurance QC range results for azithromycin, clarithromycin, and erythromycin were within previously reported limits (5).

These study results to develop CEM-101 in vitro testing conditions and QC parameters/ranges demonstrated a potent activity of this new agent against possible species causing CABP or uSSSI, such as staphylococci, streptococci, H. influenzae, and other bacteria.
enae, and E. faecalis (1, 7–16). These data confirm data from previous reports (7, 8, 11, 12, 14) and show the advantages of this novel class and similar ketolides (cethromycin and telithromycin) against pathogens that may be resistant to older macrolide-lincosamide-streptogramin B (MLSB) agents (17). We conclude that CEM-101 can be tested with confidence by CLSI methods (2–5) to detect potentially susceptible and resistant organisms. Also, the test results will be minimally influenced by departures from standardized test conditions; disk diffusion tests with 15-μg concentrations provide an optimal recognition of resistance phenotypes, and MIC testing quality can be ensured by the QC ranges proposed for the four ATCC strains studied.

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