**Introduction:**

Sulfonamides are usually the treatment of choice for *Nocardia* infections. Central nervous system and disseminated nocardiosis, however, continue to be difficult to manage. Drug resistant species of *Nocardia* such as *N. farcinica* or *N. otitidiscaviarum*, and sulfonamide intolerance, especially in patients with AIDS, require the use of alternative drugs, alone or in combination. Although clarithromycin (CLA) is not the treatment of choice in nocardiosis, promising reports of its effectiveness have been published in cases of bacteremia and pneumonia. However, clinical experience is still limited.

In this study, the activity of CEM-101, a new ketolide (Figure 1A), was evaluated in vitro compared to CLA, a structurally related macrolide (Figure 1B), against *Nocardia* spp.

CLA, obtained from Abbott Laboratories (Abbott Park, IL), was dissolved in DMSO at a concentration of 1 mg/mL. CEM-101, provided by Cempra Pharmaceuticals Inc. (Chapel Hill, NC), was dissolved in double distilled water with 3% glacial acetic acid at a concentration of 1 mg/mL. Thirty one isolates of *Nocardia*, belonging to seven species, were obtained from the American Type Culture Collection (Manassas, VA), Barbara Body, and Betty Ann Forbes. Isolates were grown in cation supplemented Mueller-Hinton (MHcs) broth and plated in MH broth to yield a standard turbidity of 100 Klett units per mL (1.2x10^6-6.0x10^5 CFU/mL). An in vitro broth microdilution method similar to that recommended by the Clinical and Laboratory Standards Institute (CLSI) was utilized for susceptibility testing.

Polystyrene 96-well round bottom plates (Corning Inc., Corning, NY) were prepared with 50 μl of MHcs broth microdilution wells. The drugs prepared at 4 X the maximal concentration tested (512 μg/ml) were added to the first well and then serially 2-fold diluted. The frozen bacterial cultures were thawed and diluted to a final concentration of about 1 x 10^6 CFU/ml in MHcs broth. The actual inoculum was measured by titration and plating on MHcs agar. To each well 50 μl of the working stock was added. The microtiter plates were covered with SealPlate adhesive sealing film (Excel Scientific, Wrightwood, CA) and incubated at 37°C in ambient air for 4-5 days prior to reading. The MIC was defined as the lowest concentration of antimicrobial agent yielding no visible turbidity. Each isolate was tested in duplicate and *Nocardia farcinica* 1 was used as a quality control in every experiment.

The results obtained for the 31 isolates tested are shown in Table 1. The end-points were generally sharp, with no more than one-dilution difference between duplicates. When this difference was observed, the higher MIC was reported. The MICs and MIC90 for CEM-101 were 0.062 mcg/mL and 32 mcg/mL, respectively, compared to 0.125 mcg/mL and 128 mcg/mL for CLA.

**Table 1: Minimum inhibitory concentrations (MICs, mcg/mL) of CEM-101**

<table>
<thead>
<tr>
<th>Group</th>
<th>CEM-101</th>
<th>Clarithromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall group (18)</td>
<td>0.062</td>
<td>0.125</td>
</tr>
<tr>
<td>Resistant group (18)</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Sensitive group (6)</td>
<td>0.031</td>
<td>0.015</td>
</tr>
<tr>
<td>Susceptible group (7)</td>
<td>0.031</td>
<td>0.015</td>
</tr>
</tbody>
</table>

**Table 2: MIC50 and MIC90 of the different groups of isolates**

Two groups of isolates could be clearly distinguished in terms of susceptibility. A resistant group, with MICs >=8 mcg/mL (14 isolates) and a susceptible group, with MICs <=0.25 mcg/mL (17 isolates), as shown in Table 2. There was good concordance with the activity of CEM-101 (Figure 2A) and CLA (Figure 2B) in each of these groups. Some species e.g. *N. farcinica* and *N. otitidiscaviarum* appeared to be resistant to these drugs. Other species, such as *N. asteroides* or *N. nova*, showed variable susceptibility patterns. Similar results in terms of variable *Nocardia* susceptibilities against clarithromycin had been previously reported (Eur. J. Clin. Microbiol. Infect. Dis., 2004; 23:69-70).

**Figure 1A:** Molecular structures of CEM-101 and clarithromycin

1A. CEM-101

1B. Clarithromycin

**Figure 2A:** Distribution of *Nocardia* isolates for CEM-101

**Figure 2B:** Distribution of *Nocardia* isolates for CLA

**Conclusions:**

The new ketolide CEM-101 was demonstrated to be active in vitro against *Nocardia* spp., showing equal or better activity than CLA against most of the isolates tested. CEM-101 should be further evaluated against Mycobacteria and in animal models of nocardial and mycobacterial infection to define its spectrum of activity.