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In Vitro Activity of CEM-101, a New Fluoroketolide Antibiotic, against *Chlamydia trachomatis* and *Chlamydia* (*Chlamydophila*) *pneumoniae*[▽]

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The *in vitro* activities of CEM-101, telithromycin, azithromycin, clarithromycin, and doxycycline against 10 isolates each of *Chlamydia trachomatis* and *Chlamydia* (*Chlamydophila*) *pneumoniae* were tested. The MIC at which 90% of the isolates of both *C. trachomatis* and *C. pneumoniae* were inhibited and the minimal bactericidal concentration at which 90% of the isolates were killed by CEM-101 were 0.25 μg/ml (ranges, 0.125 to 0.5 μg/ml for *C. trachomatis* and 0.25 to 1.0 μg/ml for *C. pneumoniae*).

The ketolides are a subclass of macrolides, which were designed specifically to overcome macrolide-resistant respiratory pathogens. Ketolides lack the cladinose sugar, which is replaced with a 3-ketone group. Ketolides bind to a secondary region on domain II of the 23S rRNA subunit (4). The ketolides are acid stable and have activity against a broad range of respiratory pathogens, including multiresistant pneumococci, *Haemophilus influenzae*, *Legionella* species, *Mycoplasma pneumoniae*, and *Chlamydia* (*Chlamydophila*) *pneumoniae* (4, 6, 8, 12). Currently only one ketolide, telithromycin, has approval from the Food and Drug Administration (FDA). However, after reports of rare but serious cases of hepatotoxicity and reports of visual disturbances and loss of consciousness that were associated with telithromycin, the FDA restricted its use in February 2007 (3, 10). Cethromycin, a ketolide in clinical development, has been studied for the treatment of respiratory infections, and a new drug application (NDA) for community-acquired bacterial pneumonia (CABP) is under review by FDA (4). We compared the *in vitro* activity of CEM-101, a new fluoroketolide antibiotic, to those of telithromycin, azithromycin, clarithromycin, and doxycycline against 10 isolates each of *Chlamydia trachomatis* and *C. pneumoniae*.

Isolates of *C. trachomatis* included standard isolates from the ATCC (E-BOUR, F-IC-CAL3, C-HAR32, J-UW-36, L2434, D-UW-57kx, and B-HAR-36) and clinical isolates N18 (cervical), N19 (cervical), and 7015 (infant eye). Isolates of *C. pneumoniae* tested included a reference strain (TW 183), 9 isolates from children and adults with pneumonia from the United States (AR39, T2023, T2043, W6805, CWL 029, and CM-1), an isolate from a child with pneumonia from Japan (J-21), and 2 isolates from bronchoalveolar lavage specimens from patients with human immunodeficiency virus infection and pneumonia from the United States (BAL15 and BAL16).

CEM-101, telithromycin, azithromycin, clarithromycin, and doxycycline were provided as powders, solubilized according to the manufacturers' instructions, and frozen in 1-ml aliquots of 2,048 μg/ml. Drug suspensions were made fresh each time the assay was run. Susceptibility testing of *C. pneumoniae* was performed with cycloheximide-treated HEp-2 cells grown in 96-well microtiter plates (7, 8). Each well was inoculated with 0.1 ml of the test strain diluted to yield 10^3 to 10^4 inclusion-forming units per ml; the plates were centrifuged at 1,700 × *g* for 1 h and incubated at 35°C for 1 h. Wells were then aspirated and overlaid with medium containing 1 μg/ml of cycloheximide and serial 2-fold dilutions of the test drug. After incubation at 35°C for 72 h, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the chlamydial lipopolysaccharide genus-specific antigen (Pathfinder; Bio-Rad, Redmond, WA). The MIC was the lowest antibiotic concentration at which no inclusions were seen. The minimal bactericidal concentration (MBC) was determined by aspirating the antibiotic-containing medium, washing wells twice with phosphate-buffered saline, and adding antibiotic-free medium. The infected cells were frozen at −70°C, thawed, passed onto new cells, incubated for 72 h, and then fixed and stained as described above. The MBC was the lowest antibiotic concentration that resulted in no inclusions after passage. All tests were run in duplicate.

The results for *C. trachomatis* are shown in Table 1. The *in vitro* activity of CEM-101 against *C. trachomatis* was similar to that of azithromycin but less than those of telithromycin, clarithromycin, and doxycycline. The MIC_{90} and MBC which was bactericidal against 90% of the isolates (MBC_{90}) of CEM-101 were 0.25 μg/ml. The MIC_{90}s for telithromycin, azithromycin, clarithromycin, and doxycycline were 0.06, 0.125, 0.125, and 0.06 μg/ml, respectively.

TABLE 1. Activities of CEM-101 and other antibiotics against 10 isolates of *C. trachomatis*

Drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
CEM-101	0.125–0.5	0.25	0.25	0.125–0.5	0.25
Telithromycin	0.015–0.25	0.06	0.06	0.015–0.25	0.06
Azithromycin	0.015–0.125	0.125	0.125	0.015–0.125	0.125
Clarithromycin	0.015–0.125	0.06	0.06	0.015–0.125	0.06
Doxycycline	0.015–0.06	0.06	0.06	0.015–0.06	0.06

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TABLE 2. Activities of CEM-101 and other antibiotics against 10 isolates of *C. pneumoniae*

Drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
CEM-101	0.25–1.0	0.25	0.25	0.25–1.0	0.25
Telithromycin	0.015–0.25	0.06	0.06	0.015–0.25	0.06
Azithromycin	0.015–0.125	0.125	0.125	0.015–0.125	0.125
Clarithromycin	0.015–0.125	0.06	0.06	0.015–0.125	0.06
Doxycycline	0.015–0.06	0.06	0.06	0.015–0.06	0.06

The activity of CEM-101 against *C. pneumoniae* was almost identical to its activity against *C. trachomatis* (Table 2). The MIC_{90} and MBC_{90} of CEM-101 were 0.25 μg/ml, whereas the MIC_{90}s for telithromycin, azithromycin, clarithromycin, and doxycycline were 0.06, 0.125, 0.06, and 0.06 μg/ml, respectively. By comparison, the MIC_{90} of cethromycin, has been reported as 0.015 μg/ml (11). However, *in vitro* activity may not necessarily predict microbiologic efficacy *in vivo* against *C. pneumoniae.* Although clarithromycin is 2- to 10-fold more active *in vitro* against *C. pneumoniae* than erythromycin (7), it was not more effective than erythromycin in eradicating *C. pneumoniae* from the nasopharynges of children with community-acquired pneumonia (2). We reported similar data for azithromycin in adults and children (9). CEM-101 also appears to have intracellular penetration superior to that of telithromycin, clarithromycin, and azithromycin, which might also result in higher *in vivo* efficacy despite a higher MIC_{90} *in vitro* than these other compounds (5).

CEM-101 has excellent activity against genital pathogens, specifically, genital mycoplasmas, including *Mycoplasma genitalium*, *Mycoplasma hominis*, and *Ureaplasma urealyticum*, with MICs ranging from ≤0.00003 to 0.008 μg/ml (12). CEM-101 also retained activity against two macrolide-resistant isolates of *M. pneumoniae*, with MICs of ≤0.5 μg/ml. This is of particular importance since macrolide resistance in *M. pneumoniae* is currently prevalent in Japan and China and has been reported in the United States (12). Further, in studies conducted by Beidenbach et al., in which the activity of CEM-101 against 34 strains of *Neisseria gonorrhoeae* was tested, the MICs were ≤0.25 μg/ml, indicating 4-fold-greater activity than azithromycin (1).

The results of the present *in vitro* study suggest that CEM-101 may be effective for the treatment of both sexually transmitted and community-acquired respiratory infections, including those due to *C. trachomatis* and *C. pneumoniae*.

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