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In Vitro Activity of Nemonoxacin, a Novel Nonfluorinated Quinolone Antibiotic, against *Chlamydia trachomatis* and *Chlamydia pneumoniae*

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The *in vitro* activities of nemonoxacin, levofloxacin, azithromycin, and doxycycline were tested against 10 isolates each of *Chlamydia trachomatis* and *Chlamydia pneumoniae*. The MICs at which 90% of the isolates of both *C. trachomatis* and *C. pneumoniae* were inhibited (MIC_{90}s) were 0.06 μg/ml (range, 0.03 to 0.13 μg/ml). The minimal bactericidal concentrations at which 90% of the isolates were killed by nemonoxacin (MBC_{90}s) were 0.06 μg/ml for *C. trachomatis* (range, 0.03 to 0.125 μg/ml) and 0.25 for *C. pneumoniae* (range, 0.015 to 0.5 μg/ml).

Chlamydia trachomatis infection is the most common sexually transmitted infection in the United States, causing more than 1.4 million cases of cervicitis and urethritis each year (1). *Chlamydia pneumoniae* is a frequent cause of community-acquired respiratory infections, including pneumonia and bronchitis, in adults and children (2). Quinolones have activity against a wide range of bacteria, including *Chlamydia* spp. (3). Antimicrobial activity of quinolones is achieved by inhibiting bacterial DNA gyrase and topoisomerase IV activities, which then inhibit bacterial DNA synthesis (3). Nemonoxacin (TG873870), a novel nonfluorinated quinolone, differs from fluoroquinolones in that it lacks the fluorine in the R6 positions. Resistance to nemonoxacin requires three different mutations in quinolone resistance-determining regions (QRDR) of genes encoding DNA gyrase and topoisomerase IV compared to two mutations in fluoroquinolone QRDR genes (4, 5).

Nemonoxacin has demonstrated potent antibacterial activities against a broad spectrum of Gram-positive cocci and Gram-negative bacilli (6–10). It has potency against respiratory pathogens, including penicillin and quinolone-resistant *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila* (6–9). Nemonoxacin has also been shown to be potent against genital pathogens such as *Neisseria gonorrhoeae* (9). We compared the *in vitro* activity of nemonoxacin to that of levofloxacin, azithromycin, and doxycycline against 10 isolates each of *C. trachomatis* and *C. pneumoniae*.

Isolates of *C. trachomatis* included the following standard isolates from the ATCC: D-UW-57Cx (VR-878), E-BOUR (VR-348B), F-IC-CAL3 (VR-346), H-UW-43Cx (VR-879), I-UW-12Ur (VR-880), J-UW-36Cx (VR-886), and L2-434 (VR-902B). The *C. trachomatis* isolates also included clinical isolates N18 (cervical), N19 (cervical), and 7015 (infant eye). Isolates of *C. pneumoniae* tested included four standard isolates from the ATCC, TW 183 (VR-2282), AR 39 (53592), CM-1 (VR-1360), and T 2043 (VR1355), and six isolates from bronchoalveolar lavage specimens from patients with human immunodeficiency virus infection and pneumonia from the United States (BAL 15, BAL 16, BAL 18, BAL 19, BAL 37, and BAL 62).

Nemonoxacin (Warner Chilcott, Dublin, Ireland), azithromycin (Sigma-Aldrich, St. Louis, MO, USA), levofloxacin (Sigma-Aldrich, St. Louis, MO, USA), and doxycycline (Sigma-Aldrich, St. Louis, MO, USA) were supplied as powders and solubilized according to the manufacturers' instructions. Drug suspensions were made fresh each time the assay was run. Susceptibility testing of *C. pneumoniae* and *C. trachomatis* was performed in HEp-2 cells grown in 96-well microtiter plates (11). Each well was inoculated with 0.2 ml of the test strain diluted to yield 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. The wells were then aspirated and overlaid with medium containing 1 μg/ml of cycloheximide and serial 2-fold dilutions of the test drugs. After incubation at 35°C for 72 h, the 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 MICs and MBCs for *C. trachomatis* and *C. pneumoniae* are shown in Tables 1 and 2. The MIC at which 90% of the isolates

TABLE 1 Activities of nemonoxacin and other antibiotics against 10 isolates of *C. trachomatis*

	MIC (μg/ml)			MBC (μg/ml)	
Drug	Range	50%	90%	Range	90%
Nemonoxacin	0.03–0.125	0.03	0.06	0.03–0.125	0.06
Levofloxacin	0.125–0.5	0.25	0.25	0.125–1	0.5
Doxycycline	0.03–0.25	0.06	0.125	0.03–0.25	0.125
Azithromycin	0.003–0.03	0.0075	0.015	0.007–0.03	0.015

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TABLE 2 Activities of nemonoxacin and other antibiotics against 10 isolates of *C. pneumoniae*

	MIC (μg/ml)			MBC (μg/ml)	
Drug	Range	50%	90%	Range	90%
Nemonoxacin	0.03–0.125	0.06	0.06	0.015–0.5	0.25
Levofloxacin	0.5	0.5	0.5	0.125–2	2
Doxycycline	0.06–0.125	0.125	0.125	0.25–0.5	0.5
Azithromycin	0.03–0.06	0.06	0.06	0.06–0.25	0.25

were inhibited (MIC_{90}) and MBC that killed 90% of the isolates (MBC_{90}) of nemonoxacin against *C. trachomatis* were 0.06 μg/ml, whereas the MIC_{90}s for levofloxacin, doxycycline, and azithromycin were 0.25, 0.125, and 0.015 μg/ml, respectively. The MBC_{90}s for levofloxacin, doxycycline, and azithromycin were 0.5, 0.125, and 0.015 μg/ml, respectively. The MIC_{90} of nemonoxacin against *C. pneumoniae* was 0.06 μg/ml, whereas the MIC_{90}s for levofloxacin, doxycycline, and azithromycin were 0.5, 0.125, and 0.06 μg/ml, respectively. The MBC_{90} of nemonoxacin was 0.25 μg/ml, and MIC_{90}s of levofloxacin, doxycycline, and azithromycin were 2, 0.5, and 0.25 μg/ml, respectively.

The *in vitro* activity of nemonoxacin against *C. trachomatis* was 2- to 3-fold higher than the *in vitro* activity of levofloxacin and doxycycline but 2-fold lower than that of azithromycin.

The *in vitro* activity of nemonoxacin against *C. pneumoniae* was comparable with that of levofloxacin, doxycycline, and azithromycin. However, *in vitro* activity may not necessarily predict microbiologic efficacy *in vivo* against *C. pneumoniae* (2).

Nemonoxacin has excellent activity *in vitro* and *in vivo* against respiratory pathogens, including methicillin- and vancomycin-resistant *Staphylococcus aureus*, levofloxacin-resistant and penicillin-resistant *Streptococcus pneumoniae*, and *Haemophilus influenzae* (6–9). Oral nemonoxacin, 750 mg and 500 mg given once daily for 7 days, showed high biological success rates for common bacterial pathogens and high clinical success rates for atypical pathogens of community-acquired pneumonia. Nemonoxacin was well tolerated, and no serious drug-related adverse events were observed (12, 13).

The activity of nemonoxacin was studied against 10 strains of *N. gonorrhoeae*, of which 8 were ciprofloxacin resistant. The MICs for nemonoxacin of the fluoroquinolone-resistant *N. gonorrhoeae* isolates were 0.25 to 1 μg/ml, which were 2- to 4-fold higher than the MICs for ciprofloxacin, levofloxacin, and moxifloxacin (9).

Nemonoxacin also retained activity against clinical isolates of members of the family *Enterobacteriaceae*, various *Nocardia* species, and *Helicobacter pylori*, but not *Mycobacterium tuberculosis* (8, 10, 14, 15).

The results of the present *in vitro* study suggest that nemonoxacin may have a potential role in the treatment of both sexually transmitted and community-acquired respiratory infections due to *C. trachomatis* and *C. pneumoniae*.

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