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

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Running Head: Activity of Nemonoxacin against Chlamydia

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Abstract

The in vitro activities of nemonoxacin, levofloxacin, azithromycin, and doxycycline were tested against 10 isolates each of *Chlamydia trachomatis* and *Chlamydia pneumoniae*. The minimum inhibitory concentrations at which 90% of the isolates of both *C. trachomatis* and *C. pneumoniae* were inhibited (MIC90) were 0.06 μg/ml (range 0.03-0.13 μg/ml). The minimal bactericidal concentrations at which 90% of the isolates were killed by nemonoxacin (MBC90) were 0.06 μg/ml for *C. trachomatis* (range 0.03-0.125 μg/ml) and 0.25 for *C. pneumoniae* (range 0.015-0.5 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 to a wide range of bacteria, including Chlamydia spp. (3). Antimicrobial activity of quinolones is mediated through inhibit 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 3 different mutations in quinolone resistant determining regions (QRDR) of gene encoding DNA gyrase and topoisomerase IV, compare to 2 mutations of QRDR in fluoroquinolones (4,5).

Nemonoxacin has demonstrated potent antibacterial activities against 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 also has 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 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), L2-434 (VR-902B) and clinical isolates N18 (cervical), N19 (cervical), and 7015 (infant eye). Isolates of C. pneumoniae tested included 4 standard isolates from the ATCC:
TW 183 (VR-2282), AR 39 (53592), CM-1 (VR-1360), T 2043 (VR1355), and 6 isolates from bronchoalveolar lavage specimens from patients with human immunodeficiency virus infection and pneumonia from the United States (BAL15, BAL16, BAL 18, BAL 19, BAL 37, BAL 62).

Nemonoxacin (Warner Chilcott, Dublin, Ireland), azithromycin (Sigma-Aldrich, MO, USA), levofoxacin (Sigma-Aldrich, MO, USA), and doxycycline (Sigma-Aldrich, 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 $\times$ g for 1 h and incubated at 35°C for 1 h. Wells were then aspirated and overlayed 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, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the chlamydial lipopolysaccharide genus-specific antigen (Pathfinder; Biorad, Redmond, Wash). The minimum inhibitory concentration (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 4.
2. The MIC at which 90% of the isolates were inhibited (MIC₉₀) and MBC which was cidal against 90% of the isolates (MBC₉₀) of nemonoxacin against *C. trachomatis* were 0.06 μg/ml, whereas the MIC₉₀s for levofloxacin, doxycycline and azithromycin were 0.25, 0.125, and 0.015 respectively. The MBC₉₀s for levofloxacin, doxycycline, and azithromycin were 0.5, 0.125, and 0.015 μg/ml, respectively. The MIC₉₀ of nemonoxacin against *C. pneumoniae* was 0.06 μg/ml, whereas the MIC₉₀s for levofloxacin, doxycycline and azithromycin were 0.5, 0.125, and 0.06 respectively. The MBC₉₀ of nemonoxacin was 0.25 μg/ml, those 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 more active in levofloxacin and doxycycline but 2 fold less active than azithromycin.

The *in vitro* activity of nemonoxacin against *C. pneumoniae* was comparable with 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 *Streptococcal pneumoniae*, *Haemophilus influenza* (6-9). Oral nemonoxacin, 750mg and 500mg once daily for 7 days, showed high biological success rates for common bacterial pathogens and clinical success rate 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 0.25-1 μg/ml, which were 2 to 4-fold more active than ciprofloxacin, levofloxacin and moxifloxacin (9).

Nemonoxacin also retained activity against clinical isolates of Enterobacteriace, 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*. 
References


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activity of nemonoxacin, a novel nonfluorinated quinolone, against 2,440 clinical isolates.

activities of nemonoxacin (TG-873870), a novel nonfluorinated quinolone, and other

Multicenter study in Taiwan of the in vitro activities of nemonoxacin, tigecycline,
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vitro activity of nemonoxacin, tigecycline, and other antimicrobial agents against

Table 1
Activities of Nemonoxacin and Other Antibiotics against 10 Isolates of *C. trachomatis*

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (μg/mL)</th>
<th>MBC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td>Nemonoxacin</td>
<td>0.03-0.125</td>
<td>0.03</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.125-0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.03-0.25</td>
<td>0.06</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.003-0.03</td>
<td>0.0075</td>
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</table>

Table 2
Activities of Nemonoxacin and Other Antibiotics against 10 Isolates of *C. pneumoniae*

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (μg/mL)</th>
<th>MBC (μg/mL)</th>
</tr>
</thead>
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<tr>
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<tr>
<td>Nemonoxacin</td>
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</tr>
<tr>
<td>Levofloxacin</td>
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</tr>
<tr>
<td>Doxycycline</td>
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</tr>
<tr>
<td>Azithromycin</td>
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<td>0.0625</td>
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