

In Vitro Activities of Azithromycin, Clarithromycin, L-Ofloxacin, and Other Antibiotics against *Chlamydia pneumoniae*

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The in vitro susceptibilities of 11 strains of *Chlamydia pneumoniae* to azithromycin, clarithromycin, erythromycin, L-ofloxacin, and doxycycline were determined. Clarithromycin was the most active agent tested, with an MIC for 90% of strains and minimal chlamydiacidal concentration for 90% of strains of 0.03 µg/ml. The activity of azithromycin was similar to those of erythromycin and doxycycline, with MICs for 90% of strains of 0.125 to 0.25 µg/ml. However, the prolonged half-life and enhanced tissue penetration of azithromycin should allow for less frequent dosing and shorter duration of therapy than with erythromycin or clarithromycin. L-Ofloxacin had activity similar to that of ofloxacin, with MICs of 0.125 to 0.5 µg/ml. From the results of this in vitro study, azithromycin and clarithromycin appear to be effective antibiotics that may have a role in the treatment of infections due to *C. pneumoniae*.

Chlamydia pneumoniae, the newly described chlamydial species, is emerging as a frequent cause of community-acquired respiratory tract infection, including pneumonia and bronchitis (4, 5). There are limited data on the treatment of these infections, and what is available suggests that treatment with either erythromycin or doxycycline may not be very efficacious (5, 6). Azithromycin, a new azalide antibiotic, and clarithromycin, a new macrolide, have attracted interest as potential therapy for community-acquired respiratory tract infection because they are active against a wide range of pathogens and have superior pharmacokinetics and tolerance compared with erythromycin (2, 12). Preliminary studies from our laboratory with two clinical isolates of *C. pneumoniae* have demonstrated that clarithromycin and azithromycin are active in vitro against this organism (1). The quinolone antibiotics also offer potential therapy for *C. pneumoniae* infections (7). Ofloxacin is currently formulated as a racemic mixture; however, the levo isomer, L-ofloxacin, appears to be more active than the dextro form (3). The purpose of this study was to test the in vitro activities of azithromycin, clarithromycin, erythromycin, L-ofloxacin, and doxycycline against 10 recent clinical isolates of *C. pneumoniae*.

Azithromycin (Pfizer Central Research), clarithromycin (Abbott Laboratories), L-ofloxacin (Ortho Pharmaceuticals), erythromycin, and doxycycline were supplied as powders and solubilized according to the instructions of the manufacturers.

We tested 11 strains of *C. pneumoniae*: a reference strain, TW183 (Washington Research Foundation, Seattle); 9 clinical isolates from Brooklyn, BAL 15, BAL 37, T2337, T2243, T2219, T2043 (ATCC VR1355), BAL 48, BAL 62, and BAL 14; and W6805, a clinical isolate from Wisconsin.

Susceptibility testing of *C. pneumoniae* was performed in cell culture with HEP-2 cells grown in 96-well microtiter plates (10). Each well was inoculated with 0.2 ml of the organism diluted to yield 10^3 inclusion-forming units per ml

and centrifuged at $2,000 \times g$ for 1 h. The wells were then aspirated and overlaid with 0.2 ml of medium containing 1 µg of cycloheximide per ml and serial twofold 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 lipopolysaccharide genus antigen (Pathfinder Chlamydia Culture Confirmation System; Kallestad Diagnostics, Chaska, Minn.).

The MIC was the lowest antibiotic concentration at which no inclusions were seen. The minimal chlamydiacidal concentration (MCC) was determined by freezing the cultures at -70°C, then thawing them, passing the disrupted cell monolayers onto new cells, incubating them for 72 h, and then fixing and staining them as described above. The MCC was the lowest antibiotic concentration which resulted in no inclusions after passage. All tests were run in triplicate.

The MICs and MCCs for *C. pneumoniae* are shown in Table 1. Clarithromycin was the most active compound tested, with an MIC and MCC for 90% of strains of 0.03 µg/ml. The activity of azithromycin was similar to that of erythromycin and doxycycline, with MICs and MCCs of 0.06 to 0.25 µg/ml. L-ofloxacin had MICs and MCCs in the range of 0.125 to 0.5 µg/ml. As the concentration of all the antibiotics tested increased, the number and size of the inclusions decreased. These abnormal inclusions were considered nonviable if they did not grow when passed onto antibiotic-free cells.

Doxycycline and erythromycin are considered the treatment of choice for infections due to *Chlamydia trachomatis* and by extrapolation have been recommended for the treatment of infections due to *C. pneumoniae*. Several of the original cases of culture-documented *C. pneumoniae* infection reported by Grayston et al. (5) were thought to be infection with *Mycoplasma pneumoniae* and were treated with erythromycin (1 g/day for 5 to 10 days). Many of these patients did not appear to respond, as they had continuing or recurring symptoms. We have observed several patients with *C. pneumoniae* infection who have been persistently culture positive and symptomatic despite 7- to 30-day courses of doxycycline and tetracycline (6).

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TABLE 1. Activity of azithromycin, clarithromycin, L-ofloxacin, and other antibiotics against 11 strains of *C. pneumoniae*^a

Drug	MIC(μ g/ml)			MCC(μ g/ml)	
	Range	50%	90%	Range	90%
Azithromycin	0.06–0.25	0.06	0.125	0.06–0.25	0.25
Clarithromycin	0.004–0.03	0.015	0.03	0.008–0.03	0.03
Erythromycin	0.06–0.25	0.06	0.125	0.06–0.25	0.125
L-Ofloxacin	0.125–0.5	0.25	0.5	0.125–0.25	0.25
Doxycycline	0.06–0.25	0.125	0.25	0.125–0.25	0.25

^a 50% and 90%, MIC (or MCC) for 50% and 90% of strains, respectively.

Data on the in vitro susceptibility of *C. pneumoniae* are limited, in part because of the small number of clinical isolates available for testing. Welsh et al. (13) reported MICs of azithromycin for *C. pneumoniae* of 0.06 to 1 μ g/ml. They tested the prototype strain, TW183, and three clinical isolates from Seattle, Wash., and Atlanta, Ga. Treatment failure may be due to other factors, including inadequate tissue penetration and poor compliance due to side effects and prolonged duration of treatment. In this respect azithromycin and clarithromycin appear to offer potential advantages over erythromycin and doxycycline. The similar in vitro activities of azithromycin and erythromycin do not take into account azithromycin's prolonged half-life in serum and tissue (30 h in serum and \geq 5 days in tissue) (12). Azithromycin has been shown to be effective as a single 1-g dose for the treatment of uncomplicated *C. trachomatis* genital tract infection in men and nonpregnant women (9). Azithromycin and clarithromycin have excellent tissue and intracellular penetration, especially into bronchial epithelium and alveolar macrophages (2, 12). These observations suggest that both drugs may be superior to erythromycin for the treatment of respiratory infections due to *C. pneumoniae*. However, clarithromycin, despite lower MICs, will require multiple daily dosing and a longer duration of therapy compared with that of azithromycin. Clinical trials utilizing culture are clearly indicated.

The quinolones have also been demonstrated to be active against both *C. trachomatis* and *C. pneumoniae* in vitro (7, 8). Ofloxacin has an indication for the treatment of genital chlamydial infection and may be effective for respiratory infection with *C. pneumoniae* (8). Ofloxacin is a racemic mixture, but L-ofloxacin has greater antimicrobial activity

(11). In animal studies, L-ofloxacin has exhibited more potent in vivo efficacy than ofloxacin, possibly due to its higher levels in serum and tissue penetration (3). The activity in vitro was the same as we previously reported for ofloxacin (7). Prospective clinical studies would be needed to demonstrate its superiority to ofloxacin.

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