

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

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The in vitro susceptibilities of *Chlamydia pneumoniae* isolates to macrolide, tetracycline, and quinolone antibiotics were determined. Tetracycline, clarithromycin, and erythromycin had the lowest MICs in the first cell culture passage. Azithromycin required the lowest concentration for complete inhibition of inclusion formation on the second pass into antibiotic-free medium, likely reflecting its high intracellular concentrations.

Chlamydia pneumoniae is an obligately intracellular bacterium which was first isolated in association with acute respiratory infection in 1986 (7). Since that time, it has been identified as a common cause of both upper and lower respiratory tract infections and more recently has been associated with chronic diseases such as asthma, sarcoidosis, and atherosclerosis (13). *C. pneumoniae* differs from *C. trachomatis* in its resistance to sulfonamides; however, like *C. trachomatis* and *C. psittaci*, it is susceptible in vitro to tetracycline and erythromycin (11).

Azithromycin and clarithromycin are recently developed antibiotics which have the potential to be more effective and better tolerated than erythromycin and tetracycline for the treatment of *C. pneumoniae* infections. We tested the in vitro susceptibility of multiple strains of *C. pneumoniae* to these agents as well as to the quinolones ofloxacin, sparfloxacin, and temafloxacin. Preliminary results of clarithromycin testing and completed results of ofloxacin testing have been previously reported (12, 14).

Drug susceptibility tests were done in HL cell culture by previously described methods (11). Four culture vials were inoculated with *C. pneumoniae* (2×10^4 inclusion-forming units [IFU] per vial with strains TW-183, AR-39, and AR-388; 1×10^4 IFU per vial with strains Ka-5c and Ka-66; and 1×10^3 IFU per vial with other strains). The test drug was added in serial twofold dilutions. After incubation at 35°C for 3 days, two vials were stained for inclusions with a fluorescein isothiocyanate-conjugated genus-specific monoclonal antibody, CF-2. The contents of the remaining two vials were harvested and passed into two new culture vials. The vials were again cultured without drug for 3 days and stained with fluorescent antibody for inclusion counts.

The MIC for the first pass, which was done with antibiotics, was defined as the lowest antibiotic concentration resulting in the absence of inclusions detected by fluorescent-antibody staining. For the second pass, which was done without antibiotics, two endpoints were determined. The MIC for 99.9% inhibition on the second pass was defined as the lowest concentration at which there was a 99.9% inhibition of inclusion formation compared with controls. The MIC for 100% inhibition on the second pass was defined by the complete absence of inclusions.

All antibiotics were tested against three standard strains of

C. pneumoniae (TW-183, AR-39, and AR-388). Some of the antibiotics were also tested against additional strains as follows: clarithromycin and 14-hydroxyclearithromycin against Ka-5c and Ka-66 (4); erythromycin against Ka-5c (4); and azithromycin against CWL-11 (1), CWL-50 (1), NY-2023 (2), Ka-5c (4), Ka-66 (4), AC-5 (18), and AC-21 (18).

Clarithromycin, 14-hydroxyclearithromycin, temafloxacin, and erythromycin lactobionate were supplied by Abbott Laboratories, North Chicago, Ill.; azithromycin and tetracycline were supplied by Pfizer Central Research, Groton, Conn.; and sparfloxacin was supplied by Parke-Davis, Ann Arbor, Mich.

The lowest first-pass MICs were seen with clarithromycin, tetracycline, and erythromycin (Table 1). For these antibiotics, the 99.9% second-pass MIC was comparable to the first-pass MIC but was between 1 and 2 log units lower than the 100% second-pass MIC. Azithromycin had a higher first-pass MIC than the three other antibiotics listed above; however, of all the antibiotics tested, azithromycin had the lowest 100% second-pass MIC and the lowest 100%/99.9% MIC ratio for second-pass inhibition.

Sparfloxacin was the most active quinolone tested against *C. pneumoniae* and was close to the macrolides in activity. High first- and 100% second-pass MICs were seen with erythromycin, tetracycline, and the quinolones.

This in vitro test method gave substantial variability from test to test, as much as 1 to 2 log units, as shown by the range of results in Table 1. However, there was no consistent difference in the patterns of susceptibilities of different *C. pneumoniae* strains.

Since our first report on the in vitro drug susceptibility testing of *C. pneumoniae* in 1988 (11), susceptibility testing has focused on the tetracyclines, macrolides, and quinolones. Our results indicate that, of those groups, *C. pneumoniae* is most susceptible to the macrolides and tetracyclines. Our results also indicate the importance of performing a second pass into antibiotic-free medium to assess in vitro susceptibility. In vivo, antibiotics must both be lethal to the metabolically active intracellular reticulate body and then maintain inhibitory levels in tissue long enough to prevent previously formed elementary bodies from causing new cycles of infection. Some drugs, such as the beta-lactams (11), inhibit the production of infectious particles by inhibition of the maturation of reticulate bodies to elementary bodies but do not completely inhibit the replication of reticulate bodies. Therefore, although relatively low MICs are seen on the second pass, much higher concentrations of drug are required to produce the complete absence of inclusions on the first pass.

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TABLE 1. Inhibition of *C. pneumoniae* inclusion formation in vitro

Antibiotic	No. of tests performed	Mean MIC (range) in $\mu\text{g/ml}$			100%/99.9% MIC ratio
		First pass with antibiotic	Second pass without antibiotic		
			99.9%	100%	
Azithromycin	10	0.22 (0.125–0.5)	0.20 (0.1–0.5)	0.29 (0.125–0.5)	1.5
Clarithromycin	13	0.04 (0.025–0.1)	0.03 (0.0125–0.1)	0.57 (0.05–2.0)	19
14-Hydroxycyclarithromycin	10	0.28 (0.05–0.5)	0.05 (0.0125–0.1)	0.45 (0.05–1.0)	9
Sparfloxacin	9	0.21 (0.05–0.5)	0.02 (0.01–0.05)	1.0 (0.1–2.0)	50
Temafloxacin	10	2.4 (2.0–4.0)	≤ 0.5	18.8 (2.0–40)	38
Ofloxacin ^a	4	1.3 (1.0–2.0)		1.3 (1.0–2.0)	
Tetracycline	4	0.08 (0.05–0.1)	0.07 (0.05–0.1)	2.8 (1.0–4.0)	40
Erythromycin	4	0.08 (0.05–0.1)	0.02 (0.01–0.05)	2.0 (0.5–4.0)	100

^a Data were published previously (14).

Of all the antibiotics we tested, azithromycin showed the highest degree of activity in providing sustained inhibition of chlamydial growth on the second pass into antibiotic-free medium. Azithromycin, 14-hydroxycyclarithromycin, and ofloxacin were the only antibiotics without significant differences in MIC between the first and second passes. However, azithromycin is more effective in killing the remaining 0.1% of the organisms. This is likely to be due to the extremely high concentrations in tissue achieved by azithromycin. Similarly, the low 100%/99.9% azithromycin MIC ratio for elimination of inclusions on the second pass also likely reflects high concentrations of the drug in tissue. The relatively low concentrations of azithromycin required for 100% elimination of inclusions on the second pass may be considered analogous to a bactericidal effect, as observed with traditional broth dilution antimicrobial susceptibility testing. Although azithromycin, which binds to the bacterial 50S ribosomal subunit and inhibits RNA-dependent protein synthesis, is, like other macrolides, generally considered to be bacteriostatic, bactericidal activity has been demonstrated in vitro against other bacterial species (5, 6, 17). Treatment failures in cases of chronic chlamydial infection are common. Therefore, the ability of an antibiotic to eradicate the residual 0.1% of the organisms may indicate whether an antibiotic is bacteriostatic or bactericidal.

Our results are in general consistent with those reported by other investigators (3, 9, 16). We did find MICs of sparfloxacin higher than those generally previously reported, however. Nakata et al. found that the sparfloxacin MICs were 0.031 $\mu\text{g/ml}$ for the first pass and 0.063 $\mu\text{g/ml}$ for the second pass against two strains of *C. pneumoniae* (15). Hammerschlag et al. reported a range of MICs on the first pass of 0.06 to 0.25 $\mu\text{g/ml}$, similar to our results; however, on the second pass they found much lower MICs than we did, with a range of 0.06 to 0.25 $\mu\text{g/ml}$ (8). Kimura et al. found an MIC on the first pass of 0.063 $\mu\text{g/ml}$ (10). However, Cooper et al. noted higher MICs more consistent with our results, with an MIC of sparfloxacin on the first pass of 0.5 $\mu\text{g/ml}$ and an MIC on the second pass of >2 $\mu\text{g/ml}$ (3).

Further testing of azithromycin and clarithromycin in a clinical setting is needed. However, they are likely to be at least as effective as and better tolerated than erythromycin and tetracycline for the treatment of respiratory infections due to *C. pneumoniae*.

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REFERENCES

- Campbell, J. F., R. C. Barnes, P. E. Kozarsky, and J. S. Spika. 1991. Culture-confirmed pneumonia due to *Chlamydia pneumoniae*. *J. Infect. Dis.* **164**:411–413.
- Chirgwin, K., P. M. Roblin, M. Gelling, M. R. Hammerschlag, and J. Schachter. 1991. Infection with *Chlamydia pneumoniae* in Brooklyn. *J. Infect. Dis.* **163**:757–761.
- Cooper, M. A., D. Baldwin, R. S. Matthews, J. M. Andrews, and R. Wise. 1991. In-vitro susceptibility of *Chlamydia pneumoniae* (TWAR) to seven antibiotics. *J. Antimicrob. Chemother.* **28**:407–413.
- Ekman, M.-R., J. T. Grayston, R. Visakorpi, M. Kleemola, C.-C. Kuo, and P. Saikku. 1993. An epidemic of infections due to *Chlamydia pneumoniae* in military conscripts. *Clin. Infect. Dis.* **17**:420–425.
- Fernandes, P. B., R. Bailer, R. Swanson, C. W. Hanson, E. McDonald, N. Ramer, D. Hardy, N. Shipkowitz, R. R. Bower, and E. Gade. 1986. In vitro and in vivo evaluation of A-56268 (TE-031), a new macrolide. *Antimicrob. Agents Chemother.* **30**:865–873.
- Goldstein, F. W., M. F. Emirian, A. Coutrot, and J. F. Acar. 1990. Bacteriostatic and bactericidal activity of azithromycin against *Haemophilus influenzae*. *J. Antimicrob. Chemother.* **25**(Suppl. A):25–28.
- Grayston, J. T., C.-C. Kuo, S.-P. Wang, and J. Altman. 1986. A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infection. *N. Engl. J. Med.* **315**:161–168.
- Hammerschlag, M. R., C. L. Hyman, and P. M. Roblin. 1992. In vitro activities of five quinolones against *Chlamydia pneumoniae*. *Antimicrob. Agents Chemother.* **36**:682–683.
- Hammerschlag, M. R., K. K. Qumei, and P. M. Roblin. 1992. In vitro activities of azithromycin, clarithromycin, l-ofloxacin, and other antibiotics against *Chlamydia pneumoniae*. *Antimicrob. Agents Chemother.* **36**:1573–1574.
- Kimura, M., T. Kishimoto, Y. Niki, and R. Soejima. 1993. In vitro and in vivo antichlamydia activities of newly developed quinolone antimicrobial agents. *Antimicrob. Agents Chemother.* **37**:801–803.
- Kuo, C.-C., and J. T. Grayston. 1988. In vitro drug susceptibility of *Chlamydia* sp. strain TWAR. *Antimicrob. Agents Chemother.* **32**:257–258.
- Kuo, C.-C., and J. T. Grayston. 1991. In vitro susceptibility of *Chlamydia pneumoniae*, strain TWAR, to clarithromycin. In Proceedings of the 17th International Congress of Chemotherapy.
- Kuo, C.-C., L. A. Jackson, L. A. Campbell, and J. T. Grayston. 1995. *Chlamydia pneumoniae* (TWAR). *Clin. Microbiol. Rev.* **8**:451–461.
- Lipsky, B. A., K. J. Tack, S.-P. Wang, C.-C. Kuo, and J. T. Grayston. 1990. Ofloxacin treatment of *Chlamydia pneumoniae* (strain TWAR) lower respiratory tract infections. *Am. J. Med.* **89**:722–724.
- Nakata, K., Y. Okazaki, H. Hattori, and S. Nakamura. 1994. Protective effects of sparfloxacin in experimental pneumonia caused by *Chlamydia pneumoniae* in leukopenic mice. *Antimicrob. Agents Chemother.* **38**:1757–1762.
- Niki, Y., M. Kimura, N. Miyashita, and R. Soejima. 1994. In vitro and in vivo activities of azithromycin, a new azalide antibiotic, against chlamydia. *Antimicrob. Agents Chemother.* **38**:2296–2299.
- Retsema, J., A. Girard, W. Schelkly, M. Manousos, M. Anderson, G. Bright, R. Borovoy, L. Brennan, and R. Mason. 1987. Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. *Antimicrob. Agents Chemother.* **31**:1939–1947.
- Yamazaki, T., H. Nakada, N. Sakurai, C.-C. Kuo, S.-P. Wang, and J. T. Grayston. 1990. Transmission of *Chlamydia pneumoniae* in young children in a Japanese family. *J. Infect. Dis.* **162**:1390–1392.