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Antibacterial Effects of Levofloxacin, Erythromycin, and Rifampin in a Human Monocyte System against *Legionella pneumophila*

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The antibacterial activities of levofloxacin, erythromycin, and rifampin against intracellular *Legionella pneumophila* L-1033, serogroup 1, were studied. In an in vitro system utilizing adherent human monocytes, *L. pneumophila* L-1033, a phagocytosis time period of 1 h, and antibiotic (levofloxacin, erythromycin, and/or rifampin) at 1 to 10 times the MIC, the CFU/ml values for the monocyte lysate were determined during 0- to 4-day time periods. The decrease in CFU/ml with levofloxacin at pH 7.4 was rapid, occurring within 24 h, and was drug concentration dependent ($P < 0.01$). The decrease in CFU with rifampin was first observed at 48 h ($P < 0.01$), while only a minimal decrease in CFU/ml was observed with erythromycin. Combination of levofloxacin and rifampin and of levofloxacin and erythromycin at ten times their MICs significantly decreased the CFU/ml value ($P < 0.01$), to the value attained by levofloxacin alone, while combination of rifampin and erythromycin did not. Removal of levofloxacin after 24 h of incubation resulted in regrowth of *L. pneumophila* L-1033, while a continued slow decrease in CFU/ml was seen following rifampin removal; CFU/ml values were unaffected by the removal of erythromycin. At 4 days, and even in assays performed following antibiotic removal, the CFU/ml value continued to be lower in the levofloxacin and rifampin assays than in the assays with erythromycin. Levofloxacin had a significantly higher bactericidal activity against *L. pneumophila* L-1033 than erythromycin or rifampin. In these assays, the addition of erythromycin or rifampin did not affect the antibacterial activity of levofloxacin.

Intracellular infections caused by bacterial pathogens are common. They can cause serious disease in compromised hosts, may relapse, and can be difficult to treat in spite of apparent in vitro susceptibilities of the pathogen to appropriate antibiotics (13, 14, 17). Legionellosis is one example of such serious intracellular infections (11, 15, 17). Because *Legionella pneumophila* causes intracellular infection, the antimicrobial agent of choice must penetrate the target cell, reach the site of bacterial replication, and exert its antimicrobial effect on the intracellular organism (25, 26).

The treatment of Legionnaires' disease to date has included erythromycin as the first-choice antimicrobial agent, followed by newer macrolides, doxycycline, or trimethoprim-sulfamethoxazole (7, 8). Fluoroquinolones have been found to be active against *L. pneumophila* in vitro in more recent studies, including studies performed with cell-associated organisms (1, 3–5, 8, 12, 19, 21). Furthermore, rifampin is commonly used as an adjunct drug in seriously ill patients with *L. pneumophila* infections (8). This is done to maximize clinical efficacy and minimize the selection of resistant mutants, although the development of resistance to rifampin could not be shown in a guinea pig model of Legionnaires' disease (6).

Levofloxacin is the more active isomer of the two optically active isomers of ofloxacin (10). Its DNA gyrase activity is similar to that of ciprofloxacin. Concentrations of levofloxacin in serum and tissues exceed those of ciprofloxacin, and the half-life of levofloxacin is longer, allowing daily single-dose administration in many cases (24). Studies of the in vitro activity of levofloxacin against *L. pneumophila* have demonstrated that this new fluoroquinolone is more active than erythromycin or ofloxacin (1). Furthermore, recent observations have demonstrated that the intracellular concentration of levofloxacin is at least six times the extracellular concentration (20).

This study was performed to define the ability of levofloxacin to enhance the inhibitory or bactericidal activity of human monocytes against *L. pneumophila* and to compare the efficacy of levofloxacin against intracellular *L. pneumophila* with those of erythromycin and rifampin, used singly or in combination.

(This work was presented in part at the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 15 to 18 September 1996 [1a].)

MATERIALS AND METHODS

Preparation of human monocytes. Monocytes were prepared from heparinized blood of healthy human donors who had signed the informed-consent form approved by the Institutional Review Board of the Albany Medical College/Stratton VA Medical Center, Albany, N.Y. Mononuclear cells were separated from whole blood by using Histopaque 1077 (Sigma, St. Louis, Mo.), giving a 98% pure mononuclear cell preparation. The separated cells were resuspended in Hanks balanced salt solution with 20% fetal calf serum (FCS) to a concentration of 2×10^6/ml. Cell viability before and after exposure to antibiotics in the control and in the phagocytic system was ≥98% as determined by the trypan blue exclusion test.

Bacterial strain. *L. pneumophila* L-1033, serogroup 1, isolated from the sputum of a patient with pneumonia, was obtained from the Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany. The isolate was kept frozen in skim milk at −70°C prior to the experiment; it was then subcultured on buffered charcoal yeast extract (BCYE) agar supplemented with 5% α-ketoglutarate (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 35°C. Prior to each experiment, colonies from a 48-h culture were subcultured from BCYE agar into BYE broth and incubated for 18 h at 35°C in a shaking water bath. The bacteria were then diluted to 10^7 CFU/ml in RPMI 1640 plus 20% FCS and kept at 4°C until their addition to the adherent phagocytic cells. Confirmation of this final bacterial concentration (in CFU per milliliter) was done by duplicate counts of bacteria cultured on BCYE agar.

Antimicrobial agents. Levofloxacin was a gift from the R. W. Johnson Research Institute. Erythromycin and rifampin were obtained from Sigma. All

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TABLE 1. The effect of levofloxacin, rifampin, and erythromycin used singly at their MICs and at two and four times their MICs against intracellular *L. pneumophila* L-1033 (pH 7.4)

Antibiotic	Antibiotic concn(s) (× MIC)	Antibiotic concn (μg/ml)	Bacterial killing (log_{10} CFU/ml)[a] on assay day:			
			1	2	3	4
None (control)			0.85	0.70	0.39	−0.02
Levofloxacin	1	0.03	−0.21	−0.68	−0.96	−1.23
	2, 4	0.06, 0.12	−0.68	−1.10	−1.29	−1.60
Rifampin	1, 2, 4	0.001, 0.002, 0.004	0.39	−0.11	−0.41	−1.04
Erythromycin	1, 2, 4	0.5, 1.0, 2.0	0.10	0.01	0.03	−0.09

[a] Values are bacterial counts (log_{10} CFU/ml) of lysed monocytes on days 1 to 4 minus bacterial counts (log_{10} CFU/ml) of lysed monocytes on day 0 (i.e., immediately following the 1-h intracellular phagocytosis). A negative value indicates net killing, and a positive value indicates net growth. The SEM range for controls is 0.16 to 0.18, and that for antibiotic-containing assays is 0.15 to 0.29.

antibiotic solutions were made fresh for each experiment in accordance with the suppliers' instructions. Using the macrodilution technique, the MIC (in micrograms per milliliter) for each drug was determined in BYE broth (16, 18). The MICs of levofloxacin, erythromycin, and rifampin for *L. pneumophila* L-1033 were 0.03, 0.5, and 0.001 μg/ml, respectively.

Opsonization. Pooled heat-inactivated normal human serum obtained from four donors was diluted to 20% (vol/vol) in RPMI 1640 and was used to opsonize *L. pneumophila* L-1033 at 35°C for 1 h.

Study design. Human mononuclear cells at a density of 2×10^6/ml were delivered in a 1-ml volume and allowed to adhere to the wells of 24-well plates (Corning/Costar Corp., Cambridge, Mass.) for 2.5 h. Monocytes adhered to the wells in a contiguous layer. Medium and nonadherent cells, including lymphocytes, were aspirated from the wells. The adherent monocyte layer was gently washed once. Opsonized *L. pneumophila* L-1033 in RPMI 1640 plus 20% FCS (10^7 cells; 1-ml volume) was added to the wells. The time period allotted for phagocytosis was 1 h. Unphagocytized bacteria were removed by aspiration, and the cell layer was washed once with RPMI 1640. Antibiotics were added to duplicate wells singly at their MICs and at 2, 4, and 10 times their MICs, and they were added in combination at 10 times their MICs or attainable serum drug concentrations. Thus, the antibiotic concentrations used in the assays were as follows: levofloxacin at 0.03, 0.06, 0.12, 0.3, and 5.0 μg/ml; rifampin at 0.001, 0.002, 0.004, 0.01, and 8.0 μg/ml; and erythromycin at 0.5, 1.0, 2.0, 5.0, and 10.0 μg/ml. Following incubation of the plates at 35°C for 0, 24, 48, 72, or 96 h, the supernatant was removed, monocytes were lysed with distilled H_2O, and the lysate was quantitatively plated in duplicate on BCYE agar. The plates were incubated for 48 h at 35°C, colonies counted, and the numbers of surviving bacteria were determined. A subset of experiments involved the removal of the antibiotic from the wells 24 h after its addition. The wells were then sampled at 48, 72, and 96 h as described above. Another subset of experiments was performed at pH 6.0. The medium (RPMI 1640 plus 20% FCS) was adjusted to pH 6.0 with 1 N HCl. This medium was used for the final suspension of monocytes and bacteria in the experiment. All experiments at pH 7.4 and 6.0 were performed three times.

Statistical analysis utilized the analysis of variance methodology (23). Analysis was done on the bacterial counts (log_{10} CFU per milliliter) of lysed monocytes on days 1 to 4 (see Table 3 for days 2 to 4) minus the bacterial counts (log_{10} CFU per milliliter) of lysed monocytes on day 0 (i.e., immediately following 1 h of phagocytosis). The standard errors of the means in Tables 1 to 4 were calculated by pooling like variances and using the expected mean squares of the analysis of variance. The level of significance was 0.05. Null hypotheses were specified a priori.

RESULTS

Table 1 demonstrated the effects of levofloxacin, rifampin, and erythromycin, used singly (at their MICs and at two and four times their MICs), in the monocyte bactericidal assay at pH 7.4 and 35°C. Each of the three antimicrobial agents demonstrated a significant decrease in CFU per milliliter compared to that of the control ($P < 0.01$). While results for the study using levofloxacin at the MIC were different from those of that drug at two and four times the MIC ($P < 0.05$), there was no statistically significant difference in the results with rifampin or erythromycin; therefore, the data for studies at the MIC and at two and four times the MICs for erythromycin and for rifampin were combined. At the MIC, levofloxacin demonstrated significantly greater bactericidal activity than rifampin or erythromycin ($P < 0.01$). There was no statistically significant difference in the results of studies with levofloxacin at two and four times the MIC; therefore, these data were combined.

Table 2 demonstrates the effects of levofloxacin, rifampin, and erythromycin (at 10 times their MICs), used singly and in combination, in the monocyte bactericidal assay at pH 7.4 and 35°C. Levofloxacin demonstrated the most rapid and effective activity in decreasing the CFU per milliliter ($P < 0.01$). There was not a statistically significant difference between the results obtained with levofloxacin alone and those obtained when levofloxacin was used in combination with rifampin or erythromycin. In contrast, the CFU-per-milliliter values obtained with erythromycin and rifampin, alone or in combination, were not statistically significantly different from those of the control or from each other.

Table 3 demonstrates the effect of levofloxacin, rifampin, and erythromycin at maximum attainable serum drug concentrations in the bactericidal human monocyte assay at pH 7.4 and 35°C. In the continued presence of antibiotics, on day 2 of the assay for levofloxacin and on day 3 for rifampin, decreases in CFU per milliliter of 3 logs or more were detected. This effect continued through day 4. In contrast, viable CFU-per-milliliter values for erythromycin were similar to those of controls on days 2 to 4. Following the removal of levofloxacin on day 1, the CFU-per-milliliter values increased on days 2 through 4 ($P < 0.01$). Following the removal of rifampin on day 1, the CFU-per-milliliter value continued to decrease, but this decrease was less than in assays in which rifampin was not removed. Furthermore, in assays in which levofloxacin or rifampin was removed, the CFU per milliliter on day 4 remained

TABLE 2. The effect of levofloxacin, rifampin, and erythromycin, at ten times their MICs, used singly and in combination against intracellular *L. pneumophila* L-1033 (pH 7.4)

Antibiotic (concn, μg/ml)	Bacterial killing (log_{10} CFU/ml)[a] on assay day:			
	1	2	3	4
None (control)	0.78	0.25	0.35	0.12
Levofloxacin (0.3)	−1.14	−1.57	−2.04	−2.42
Rifampin (0.01)	0.48	0.20	−0.44	−0.73
Erythromycin (5.0)	0.35	0.34	−0.02	0.16
Levofloxacin (0.3) plus rifampin (0.01)	−1.47	−1.73	−2.21	−2.47
Levofloxacin (0.3) plus erythromycin (5.0)	−1.17	−1.27	−1.72	−1.61
Rifampin (0.01) plus erythromycin (5.0)	0.39	0.14	−0.26	−0.16

[a] See Table 1, footnote *a*. SEM range for controls and for antibiotic-containing assays is 0.20 to 0.22.

TABLE 3. The effect of levofloxacin, rifampin, and erythromycin at maximum attainable serum drug concentrations against intracellular *L. pneumophila* L-1033 (pH 7.4)

Antibiotic	Antibiotic concn (μg/ml)	Antibiotic removed[a]	Bacterial killing ($\log_{10}$ CFU/ml)[b] on assay day:			
			1	2	3	4
Control			0.64	0.39	0.12	−0.32
Levofloxacin	5	No	−2.48	−2.96	−3.49	−4.22
	5	Yes		−1.44	−1.55	−1.35
Rifampin	8	No	−0.10	−1.45	−3.67	−4.43
	8	Yes		−0.32	−0.91	−1.15
Erythromycin	10	No	−0.04	0.07	0.02	−0.02
	10	Yes		0.12	−0.02	−0.33

[a] Antibiotics were removed from the assay on day 1, and the results were compared with controls (antibiotic not removed).
[b] See Table 1, footnote *a*. SEM range for controls is 0.16 to 0.18, and that for antibiotic-containing assays is 0.46 to 0.65.

TABLE 4. The effect of levofloxacin, rifampin, and erythromycin, at 10 times their MICs, used singly and in combination against *Legionella pneumophila* L-1033 (pH 6.0)

Antibiotic (concn, μg/ml)	Bacterial killing ($\log_{10}$ CFU/ml)[a] on assay day:			
	1	2	3	4
Control	1.13	1.23	1.41	1.07
Levofloxacin (0.3)	0.47	0.53	0.18	0.12
Rifampin (0.01)	0.40	0.35	0.36	−0.12
Erythromycin (5.0)	0.60	0.36	0.48	0.64
Levofloxacin (0.3) plus rifampin (0.01)	0.40	0.08	0.27	−0.26
Levofloxacin (0.3) plus erythromycin (5.0)	0.45	0.22	0.30	−0.06
Rifampin (0.01) plus erythromycin (5.0)	0.58	0.52	0.84	0.07

[a] See Table 1, footnote *a*. SEM range for controls and for antibiotic-containing assays is 0.22 to 0.40.

lower than that for erythromycin. For erythromycin, the CFU-per-milliliter value remained the same regardless of whether the antibiotic was removed.

Table 4 depicts the effects of levofloxacin, rifampin, and erythromycin, alone or in combination, at ten times their MICs when determined at 35°C and pH 6.0 in the bactericidal human monocyte assay. Although the corrected $\log_{10}$ CFU-per-milliliter values were lower than those of the control (days 1 through 4; $P < 0.01$), there were no statistically significant differences in the antibacterial activities of the three antibiotics, or combinations thereof, at pH 6.0. Changing the pH value from 7.4 to 6.0 decreased the effectiveness of levofloxacin but did not alter the activity of rifampin or erythromycin (Tables 2 and 4). As demonstrated in Tables 1 to 4, the corrected $\log_{10}$ CFU-per-milliliter values for the controls were higher ($P < 0.05$) on days 2 to 4 when the pH was 6.0 (Table 4) than they were at pH 7.4 (Tables 1 to 3).

DISCUSSION

Entry of *L. pneumophila* into phagocytic cells is accomplished by coiling phagocytosis. *L. pneumophila* inhibits the capacity of the phagocytic cells to form a ribosome-lined replicative phagosome, to acidify the phagosome, and to allow phagosome-lysosome fusion (14). *L. pneumophila* is known to be resistant to the bactericidal effects of serum and to the killing effect of monocytes, polymorphonuclear leukocytes, and alveolar macrophages (13, 14). Even *L. pneumophila* that has been pretreated with antibiotics is capable of multiplication within human monocytes (21).

Treatment of infections caused by *L. pneumophila* is effective only when antimicrobial agents capable of intracellular activity are used (8, 9, 11, 14, 17). Therapeutic failure is not related to the development of resistance but rather is due to the inability of the phagocytic cell to eliminate the organism from its intracellular environment or to a decreased cell-mediated immune response caused by the organism (2, 6, 13, 14, 26).

A good correlation between the intracellular penetration of specific antibiotics and the survival of patients with Legionnaires' disease has been found (8, 11, 15, 17). For many years, erythromycin has been the antimicrobial agent of choice for the treatment of Legionnaires' disease (8). More recently, the activities of fluoroquinolones and newer macrolides against *L. pneumophila* have been studied in vitro and in systems including macrophages (1, 3–5, 8, 9, 12, 19–22, 25). Our previously reported and present data support the effectiveness of levofloxacin against *L. pneumophila* (1, 21). In our present comparative study with erythromycin and rifampin, we found levofloxacin to be the most effective drug against intracellular *L. pneumophila*. A statistically significant decrease in CFU per milliliter could be demonstrated with increasing concentrations of levofloxacin (at the MIC versus two and four times the MIC), but not with rifampin or erythromycin ($P < 0.05$). Similarly, at 10 times the MIC, levofloxacin was most active singly or in combination with rifampin or erythromycin compared with erythromycin or rifampin alone or in combination ($P < 0.01$). Furthermore, when the maximum drug concentrations attainable in serum were compared, levofloxacin continued to be the most active drug ($P < 0.01$) for the entire assay time (4 days). Removal of levofloxacin or rifampin from the human monocyte system on day 1 was associated with regrowth of the organism on days 2 through 4, but the CFU-per-milliliter values continued to remain lower than those for erythromycin. This observation suggests that even with a falling intracellular levofloxacin concentration after removal of the drug from the surroundings, the continued suppression of *L. pneumophila* L-1033 growth was greater than that observed with erythromycin. For erythromycin, the percent viable count remained the same regardless of whether the antibiotic was removed. These observations were best demonstrated at pH 7.4. By the trypan blue exclusion test, it was determined that >90% of the adherent monocytes survived at pH 6 during the 4 days of the assay. Recently, we have demonstrated that levofloxacin is concentrated in adherent monocytes and that its concentrations at pH 7.4 and 6 are similar (20). The reason why the activities of all three antibiotics studied were similar at pH 6 requires further study.

As noted in a recent review of antimicrobial therapy for Legionnaires' disease, small numbers of patients have been successfully treated with fluoroquinolones, including ofloxacin, pefloxacin, and ciprofloxacin (8). Although rifampin is frequently used concomitantly with erythromycin or fluoroquinolones, to date there is no published clinical evidence that such combinations are more effective than single-drug therapy. Our study failed to demonstrate better intracellular killing when rifampin was used in combination with either levofloxacin or erythromycin. Furthermore, combinations of levofloxacin and either erythromycin or rifampin were no better than levofloxacin alone in our intracellular human monocyte system.

In conclusion, our previous in vitro studies and the present

intracellular bactericidal data obtained in studies utilizing the human monocyte system demonstrate that levofloxacin is a considerably more active antimicrobial agent than erythromycin or rifampin against *L. pneumophila*. Its use for treatment of patients with Legionnaires' disease should be considered.

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REFERENCES

1. **Baltch, A. L., R. P. Smith, and W. Ritz.** 1995. Inhibitory and bactericidal activities of levofloxacin, ofloxacin, erythromycin, and rifampin used singly and in combination against *Legionella pneumophila*. Antimicrob. Agents Chemother. **39:**1661–1666.

1a. **Baltch, A. L., R. P. Smith, M. Franke, and P. Michelsen.** 1996. Antibacterial effect of levofloxacin (Lev), erythromycin (Ery) & rifampin (Rif) in a human monocyte (HM) system against *Legionella pneumophila* (L.pn.), abstr. E67, p. 93. *In* Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

2. **Barker, J., and M. R. W. Brown.** 1995. Speculations on the influence of infecting phenotype on virulence and antibiotic susceptibility of *Legionella pneumophila*. J. Antimicrob. Chemother. **36:**7–21.

3. **Barker, J. E., and I. D. Farrell.** 1990. The effects of single and combined antibiotics on the growth of *Legionella pneumophila* using time-kill studies. J. Antimicrob. Chemother. **26:**45–53.

4. **Dubois, J., and J. R. Joly.** 1989. In vitro activity of lomefloxacin (SC47111 or NY-198) against isolates of *Legionella* spp. Diagn. Microbiol. Infect. Dis. **12:**89S–91S.

5. **Edelstein, P. H., M. A. C. Edelstein, J. Weidenfeld, and M. B. Dorr.** 1990. In vitro activity of sparfloxacin (CI-978; AT-4140) for clinical *Legionella* isolates, pharmacokinetics in guinea pigs, and use to treat guinea pigs with *L. pneumophila* pneumonia. Antimicrob. Agents Chemother. **34:**2122–2127.

6. **Edelstein, P. H.** 1991. Rifampin resistance of *Legionella pneumophila* is not increased during therapy for experimental Legionnaires disease: study of rifampin resistance using a guinea pig model of Legionnaires disease. Antimicrob. Agents Chemother. **35:**5–9.

7. **Edelstein, P. H.** 1993. Legionnaires' disease. Clin. Infect. Dis. **16:**741–749.

8. **Edelstein, P. H.** 1995. Antimicrobial therapy for Legionnaires' disease: a review. Clin. Infect. Dis. **21**(Suppl. 3)**:**S8265–S8276.

9. **Edelstein, P. H., M. A. C. Edelstein, K. H. Lehr, and J. Ren.** 1996. In vitro activity of levofloxacin against clinical isolates of *Legionella* spp., its pharmacokinetics in guinea pigs, and use in experimental *Legionella pneumophila* pneumonia. J. Antimicrob. Chemother. **37:**117–127.

10. **Fu, K. P., S. C. Lafredo, B. Foleno, D. M. Isaacson, J. F. Barrett, A. J. Tobia, and M. E. Rosenthale.** 1992. In vitro and in vivo antibacterial activities of levofloxacin (*l*-ofloxacin), an optically active ofloxacin. Antimicrob. Agents Chemother. **36:**860–866.

11. **Gump, D. W., R. O. Frank, W. C. Foster, C. V. Broome, and W. B. Cherry.** 1979. Legionnaires' disease in patients with associated serious disease. Ann. Intern. Med. **90:**538–542.

12. **Havlichek, D., L. Saravolatz, and D. Pohlod.** 1987. Effect of quinolones and other antimicrobial agents on cell-associated *Legionella pneumophila*. Antimicrob. Agents Chemother. **31:**1529–1534.

13. **Horwitz, M. A.** 1982. Phagocytosis of microorganisms. Rev. Infect. Dis. **4:**104–123.

14. **Horwitz, M. A.** 1987. Characterization of avirulent mutant *Legionella pneumophila* that survive but do not multiply within human monocytes. J. Exp. Med. **166:**1310–1328.

15. **Kirby, B. D., K. M. Snyder, R. D. Meyer, and S. M. Finegold.** 1980. Legionnaires' disease: report of 65 nosocomially acquired cases and review of the literature. Medicine (Baltimore) **59:**188–205.

16. **Liebers, D. M., A. L. Baltch, R. P. Smith, M. C. Hammer, and J. V. Conroy.** 1989. Susceptibility of *Legionella pneumophila* to eight antimicrobial agents including four macrolides under different assay conditions. J. Antimicrob. Chemother. **23:**37–41.

17. **Morley, J. N., L. C. Smith, A. L. Baltch, and R. P. Smith.** 1994. Recurrent infection due to *Legionella pneumophila* in a patient with AIDS. Clin. Infect. Dis. **19:**1130–1132.

18. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.

19. **Reda, C., T. Quaresima, and M. C. Pastoris.** 1994. In vitro activity of six intracellular antibiotics against *Legionella pneumophila* strains of human and environmental origin. J. Antimicrob. Chemother. **33:**757–764.

20. **Smith, R. P., A. L. Baltch, M. A. Franke, and W. Ritz.** 1996. Levofloxacin (LOF) is concentrated in human monocytes (MN) and augments MN killing of cell associated (CA) *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA), abstr. D-91, p. 258. *In* Abstracts of the 96th General Meeting of the American Society for Microbiology 1996. American Society for Microbiology, Washington, D.C.

21. **Smith, R. P., A. L. Baltch, M. Franke, W. Hioe, W. Ritz, and P. Michelsen.** 1997. Effect of levofloxacin, erythromycin or rifampin pretreatment on growth of *Legionella pneumophila* in human monocytes. J. Antimicrob. Chemother. **40:**673–678.

22. **Stamler, D. A., M. A. C. Edelstein, and P. H. Edelstein.** 1994. Azithromycin pharmacokinetics and intracellular concentrations in *Legionella pneumophila*-infected and uninfected guinea pigs and their alveolar macrophages. Antimicrob. Agents Chemother. **38:**217–222.

23. **Stuart, A., and J. K. Ord.** 1991. Kendall's advanced theory of statistics, vol. 2, p. 1101–1153. Oxford University Press, New York, N.Y.

24. **Takasuma, K., Y. Kasai, C. Usui, M. Takahashi, M. Hirohashi, K. Tamura, and S. Takayama.** 1992. General pharmacology of the new quinolone antibacterial agent levofloxacin. Drug Res. **42:**408–418.

25. **Vildé, J. L., E. Dournon, and P. Rajagopalan.** 1986. Inhibition of *Legionella pneumophila* multiplication within human macrophages by antimicrobial agents. Antimicrob. Agents Chemother. **30:**743–748.

26. **Yourtee, E. L., and R. K. Root.** 1984. Effects of antibiotics on phagocyte-microbe interaction, p. 243–275. *In* R. K. Root and M. A. Sande (ed.), New dimensions in antimicrobial therapy. Churchill Livingstone, New York, N.Y.