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In Vitro Antimycoplasmal Activities of Rufloxacin and Its Metabolite MF 922

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The in vitro activities of rufloxacin and its metabolite, MF 922, were compared with those of ofloxacin, ciprofloxacin, erythromycin, and minocycline against *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, and *Ureaplasma urealyticum*. Rufloxacin, MF 922, and ciprofloxacin shared similar activities against all mycoplasmas tested. (MICs for 90% of isolates tested [MIC_{90}s], 0.5 to 4 μg/ml). Ofloxacin had the lowest MIC_{90}s for *U. urealyticum*, *M. fermentans*, and *M. hominis* (MIC_{90}s, 0.25 to 1 μg/ml) and erythromycin had the lowest MIC_{90} for *M. pneumoniae* (MIC_{90}, 0.004 μg/ml).

Fluoroquinolones represent a new class of broad-spectrum antimicrobial agents which are active against gram-negative and many gram-positive organisms. These compounds are bactericidal and generally have good pharmacokinetic and safety profiles. However, some pathogens, including mycoplasmas, are not sufficiently susceptible at low concentrations to many of the quinolones tested (1–3, 7–9, 16–18, 21, 22, 25, 27, 33, 34). Several new compounds have been developed in an attempt to broaden the spectrum of activity, increase the potency, and enhance the disposition of the drug at infection sites.

Rufloxacin (Fig. 1) is a new oral 6-fluoroquinolone with a broad spectrum of activity against gram-negative and gram-positive aerobic bacteria and some intracellular pathogens, including *Chlamydia pneumoniae* and *Legionella pneumophila* (10, 23, 36). In vitro, the antibacterial activity of rufloxacin is similar to that of norfloxacin (36), while in animal models it has activity similar to that of ciprofloxacin (12). In humans pharmacokinetic studies have demonstrated that rufloxacin is eliminated slowly, with a half-life in plasma of about 35 h (14, 19, 37). The drug penetrates well into most body fluids, tissues, and cells, where it reaches high and stable concentrations, ranging from 2 to 25 times those in plasma (4, 19, 35, 37). Particularly high concentrations are achieved in epithelial lining fluid (peak mean values, 42 μg/ml) and alveolar macrophages (peak mean values, 88 μg/ml) (35). Steady high concentrations (60 μg/ml) of rufloxacin are attained in urine up to 72 h following administration of a single 400-mg dose (37). In view of its pharmacokinetic profile, it can be used once daily for the treatment of urinary (5, 24) and respiratory tract (20) infections. It is metabolized at a low level. The *N*-desmethyl derivative (MF 922) (Fig. 1) is the only microbiologically active metabolite detected. In urine it accounts for 2% of the given dose (19). The in vitro activity of MF 922 is comparable to that of the parent compound (36). However, their effects on mycoplasmas have not been described previously.

The objective of the study described here was to determine the in vitro susceptibilities of *Mycoplasma pneumoniae*, *Mycoplasma hominis*, *Mycoplasma fermentans*, and *Ureaplasma urealyticum* to rufloxacin and MF 922 compared with those of ofloxacin, ciprofloxacin, erythromycin, and minocycline. Minocycline was selected rather than tetracycline because of its better in vitro activity against *U. urealyticum* and *M. pneumoniae* (1, 31). All strains tested were both clinical isolates and American Type Culture Collection standards. In the present study we investigated the susceptibilities of 160 freshly isolated *U. urealyticum* strains (from cervix, urethra, and vagina), 33 low-passage clinically isolated strains of *M. hominis* (from vagina, urethra, and cervix), 16 low-passage clinically isolated strains and 2 reference strains (FH and M129) of *M. pneumoniae*, and 11 low-passage strains (from vagina and urethra) and 1 reference strain (PG18) of *M. fermentans* to rufloxacin and MF 922 in comparison with those of ofloxacin, ciprofloxacin, erythromycin, and minocycline.

Rufloxacin and MF 922 were kindly provided by Mediolanum Farmaceutici (Milan, Italy). Ofloxacin was obtained from Sigma Tau (Pomezia, Italy), ciprofloxacin was from Bayer (Milan, Italy), erythromycin was from Sigma Chemicals (Milan, Italy), and minocycline was from Cyanamid (Catania, Italy). Rufloxacin, MF 922, and ofloxacin were dissolved in 5 ml of water, and 0.1 mol of NaOH per liter was added dropwise up to a final volume of 10 ml (5,120 μg/ml) to improve solubilization. Erythromycin, ciprofloxacin, and minocycline solutions were prepared by the procedures of the National Committee for Clinical Laboratory Standards (26).

Working solutions were prepared in 0.1 M phosphate buffer, and the final pH was the same as that of the assay medium.

M. pneumoniae and *M. fermentans* were grown in SP-4 broth (pH 7.5) (32). *U. urealyticum* and *M. hominis* were grown in 10-B broth (pH 6.0) (30). For *M. hominis* the urea present in the 10-B broth was replaced with 1% arginine.

The MIC was determined by a broth microdilution assay that was essentially equivalent to a metabolism inhibition test (29). Mycoplasma broth (0.025 ml of the specific broth) was inoculated into microtiter wells. The stock solution (0.025 ml) of each drug was added to the first well, and serial twofold dilutions (0.025 ml) were made with a multichannel pipette beginning with the second well; the final 0.025 ml was discarded. A total of 11 concentrations of each drug were prepared. A suspension of organisms (0.175 ml) was added to each well containing the drugs. Plates were sealed with trans-

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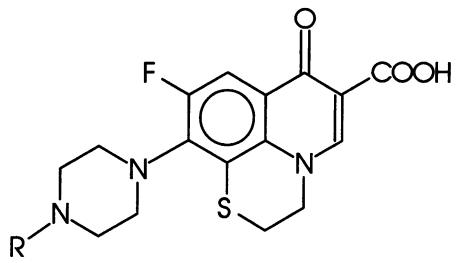

FIG. 1. Structural formulas of rufloxacin and MF 922 (for rufloxacin, R = H_3C; for MF 922, R = H).

parent acetate and were incubated at 37°C under atmospheric conditions.

Each strain was cloned three times before the test and was then used for MIC determinations. The number of organisms added was verified by making serial 10-fold dilutions in order to ensure an adequate (10^3 CFU/ml) but not an excessive ($>10^5$ CFU/ml) amount of inoculum for the test system (15, 29). All microplates were examined after 18 h of incubation and then once daily until growth in the organism control tube occurred. The MIC was defined as the lowest concentration of antibiotic that inhibited a color change in the broth by a given strain of mycoplasma at the time when the color of the control tube changed, that is, when the pH of the medium decreased from 7.5 to 7.0 (*M. pneumoniae* and *M. fermentans*) or increased from 6.0 to 6.5 (*M. hominis* and *U. urealyticum*). For *U. urealyticum*, the MIC was read after a single overnight incubation. The required incubation times were 24 to 48 h for *M. hominis* and *M. fermentans* and from 3 to 5 days for *M. pneumoniae*. Further incubations were not carried out. Each mycoplasma strain was tested six times against each antimicrobial agent. The strains were tested six additional times, on different days, with all drugs to ensure the reproducibility of the results.

A positive control (growth) consisting of organisms in broth, a negative control (sterility) consisting of uninoculated broth, and a drug control consisting of broth with the highest concentrations of drug were included for each mycoplasmal strain tested.

Staphylococcus aureus ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Escherichia coli* ATCC 25922 were included as controls; the MICs of most of the drugs tested, obtained in Mueller-Hinton broth, have been published previously (26). Each of these reference strains was inoculated into microtiter plates containing 10-B broth, SP-4 broth, Mueller-Hinton broth (BBL), and the appropriate dilutions of each antibiotic tested for MIC determinations. These control procedures were repeated each time that an assay was performed.

The results of the in vitro susceptibility tests are given in Table 1. The MICs of rufloxacin and MF 922 for the investigated strains were similar to those of ciprofloxacin but were higher than those of ofloxacin. The three quinolones tested were active against the erythromycin-resistant and minocycline-resistant mycoplasmas. Erythromycin and minocycline were the most active antibiotics tested against *M. pneumoniae* (MICs for 90% of strains tested [MIC_{90}s], 0.004 and 0.5 μg/ml, respectively), and ofloxacin was the most active among the quinolones tested (MICs, 0.12 to 0.5 μg/ml). Rufloxacin, MF 922, and ciprofloxacin showed comparable in vitro activities against *M. pneumoniae*.

The *M. hominis* strains showed the predictable uniform level of resistance to erythromycin (MICs, ≥256 μg/ml) and a moderate degree of susceptibility to minocycline (MICs, 0.12 to 16 μg/ml).

TABLE 1. Susceptibilities of *M. pneumoniae*, *M. hominis*, *M. fermentans*, and *U. urealyticum* to rufloxacin, MF 922, ofloxacin, ciprofloxacin, erythromycin, and minocycline

Organism (no. of isolates)	Drug	MIC (μg/ml)		
		Range	50%	90%
M. pneumoniae (18)	Rufloxacin	1–2	1	2
	MF 922	1–2	1	2
	Ciprofloxacin	1–2	2	2
	Ofloxacin	0.12–0.5	0.5	0.5
	Erythromycin	0.001–0.008	0.002	0.004
	Minocycline	0.06–1	0.12	0.5
M. hominis (33)	Rufloxacin	1–2	1	2
	MF 922	1–2	2	2
	Ciprofloxacin	1–2	2	2
	Ofloxacin	0.05–1	1	1
	Erythromycin	≥256		
	Minocycline	0.12–16	1	4
M. fermentans (12)	Rufloxacin	0.06–2	0.5	0.5
	MF 922	0.06–2	0.5	0.5
	Ciprofloxacin	0.06–2	0.5	0.5
	Ofloxacin	0.06–1	0.25	0.25
	Erythromycin	64–≥256	≥256	≥256
	Minocycline	0.06–1	0.25	0.5
U. urealyticum (160)	Rufloxacin	1–4	2	4
	MF 922	2–4	2	4
	Ciprofloxacin	0.5–4	2	4
	Ofloxacin	0.25–2	0.5	1
	Erythromycin	0.06–≥256	0.5	≥256
	Minocycline	0.5–16	4	8

The *M. fermentans* strains showed the expected level of resistance to erythromycin (MICs, 64 to ≥256 μg/ml) and a remarkable degree of susceptibility to minocycline (MICs, 0.06 to 1 μg/ml). Rufloxacin and MF 922 showed comparable activities against all strains of *M. hominis* and *M. fermentans* (MICs, 1 to 2 and 0.06 to 2 μg/ml, respectively). The MIC_{90}s of rufloxacin and MF 922 were equal to or lower than those of ciprofloxacin for all of the strains. The MIC_{90} of ofloxacin was 1 dilution lower.

The *U. urealyticum* isolates were inhibited by erythromycin at MICs ranging from 0.06 to ≥256 μg/ml. Of the 160 *U. urealyticum* isolates, 26 (16%) were highly resistant to erythromycin (MICs, ≥256 μg/ml). This is in agreement with the recently increasing frequency of occurrence of erythromycin-resistant *U. urealyticum* strains (33, 34). The MICs of minocycline for *U. urealyticum* were lower than those of erythromycin. The MICs of ofloxacin were 3 dilutions lower than those of minocycline and 2 dilutions lower than those of rufloxacin, MF 922, and ciprofloxacin.

Table 2 shows the acceptable MIC ranges of erythromycin, ofloxacin, ciprofloxacin, and minocycline (26) and the MICs of rufloxacin, MF 922, ciprofloxacin, ofloxacin, erythromycin, and minocycline for *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *E. coli* ATCC 25992 when they were tested in our laboratory in Mueller-Hinton broth (pH 7.2 to 7.4). The MICs obtained in SP-4 (pH 7.5) and 10-B broth (pH 6.0) are shown for comparison. SP-4 yielded MICs for *S. aureus* and *E. faecalis*, when they were tested with erythromycin and minocycline, which matched the acceptable MICs for these strains in Mueller-Hinton broth. In contrast, the MICs of erythromy-

TABLE 2. Effect of mycoplasmal media on MICs for *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *E. coli* ATCC 25922

Organism	Drug	MIC (μg/ml)			
		NCCLS range[a]	MH[b]	SP-4	10-B
S. aureus ATCC 29213	Rufloxacin	NA[c]	0.5	1	2
	MF 922	NA	8	16	16
	Ofloxacin	0.12–1	0.25	0.5	2
	Ciprofloxacin	0.12–0.5	0.5	1	2
	Erythromycin	0.12–0.5	0.12	0.06	2
	Minocycline	0.12–0.5	0.25	0.25	0.06
E. faecalis ATCC 29212	Rufloxacin	NA	2	8	8
	MF 922	NA	8	8	8
	Ofloxacin	1–4	2	8	8
	Ciprofloxacin	0.25–2	1	4	4
	Erythromycin	1–4	2	2	2
	Minocycline	2–8	2	2	2
E. coli ATCC 25922	Rufloxacin	NA	0.25	0.5	1
	MF 922	NA	0.25	0.5	1
	Ofloxacin	0.015–0.12	0.03	0.06	0.15
	Ciprofloxacin	0.004–0.015	0.008	0.015	0.03
	Erythromycin	NA	NT[d]	NT	NT
	Minocycline	0.5–2	0.5	0.5	0.5

[a] Range of acceptable MICs according to the National Committee for Clinical Laboratory Standards (NCCLS) (26).
[b] MICs obtained in our laboratory in Mueller-Hinton broth.
[c] NA, not available from the National Committee for Clinical Laboratory Standards (26).
[d] NT, not tested.

cin for *S. aureus* achieved in 10-B broth differed from those determined in Mueller-Hinton broth or SP-4 broth, being 2 dilutions higher. Moreover, with the quinolones tested SP-4 and 10-B yielded different MICs for the reference strains. These MICs were 1 or more dilutions higher than those of rufloxacin and MF 922 obtained in Mueller-Hinton broth.

Studies comparing the activities of quinolones with those of erythromycin and other macrolides against *M. pneumoniae* have shown that macrolides consistently have the lowest MICs, often by several dilutions (1, 3, 7, 8, 16, 17, 21, 25, 27, 33, 34). Fluoroquinolones may have favorable MICs, and some may perform better than others (3). Therefore, in the ranking of fluoroquinolones by Kenny and Cartwright (17), ofloxacin and ciprofloxacin were found to have similar activities against *M. pneumoniae*. If we consider this ranking, rufloxacin and MF 922 should have activities similar to those of both of these quinolones. However, the results obtained in the present study indicate that ofloxacin is twofold more active than rufloxacin, MF 922, and ciprofloxacin.

Quinolones are more active than minocycline against *M. hominis*, with ofloxacin being more potent than rufloxacin, MF 922, and ciprofloxacin. These results are comparable to those reported previously by Kenny and colleagues (17, 18). Ciprofloxacin proved to have an intermediate level of activity, according to the breakpoint (26).

Unfortunately, few studies have compared the activities of various antibiotics against *M. fermentans* (6, 8, 13, 21, 25). In the present study, *M. fermentans* was susceptible to minocycline and all quinolones tested.

U. urealyticum showed a variable and somewhat poor degree of susceptibility to quinolones and in general was less susceptible than *M. hominis* and *M. fermentans* to these agents (2, 9, 17, 18, 21, 22, 31, 33, 34). At present, few quinolones can be considered active; only two of them (sparfloxacin and WIN 57273) have MIC_{90}s of less than 1 μg/ml (17, 34). With reference to the breakpoint MICs (26, 28), our study indicated that ofloxacin alone may be considered highly active and to have potential clinical relevance, while rufloxacin, MF 922, and ciprofloxacin have intermediate activities. Nevertheless, if we compare rufloxacin with ciprofloxacin, we may say that rufloxacin has greater overall potential, given its superior kinetics in urine and tissues. The longer period that the rufloxacin concentration is greater than the MICs compared with that for ciprofloxacin and the greater areas under the concentration-time curve in urine achieved by rufloxacin compared with those achieved by ciprofloxacin probably compensate, at least in part, for the moderate degree of potency of rufloxacin in vitro (11, 28, 37). Because of its broad spectrum of activity, rufloxacin may prove to be an important drug for use in the treatment of mixed chlamydial, gonorrhoeal, and mycoplasmal genital infections. On this basis, rufloxacin is a potential candidate for the empiric treatment of sexually transmitted diseases such as nonspecific urethritis and mucopurulent cervicitis.

Macrolides continue to be the recommended treatment for mycoplasmal pneumonia, while some reluctance in the relevance of using fluoroquinolones as an alternative treatment for this pathology has been expressed (3). However, the rufloxacin concentrations in epithelial lining fluid and alveolar macrophages exceed the low breakpoint and the MIC_{90} for *M. pneumoniae* by approximately 7 and 20 times, respectively, 24 h after administration of a single 400-mg dose (35). In view of its good penetration into respiratory sites, rufloxacin may be considered a potential new drug for the treatment of *M. pneumoniae* pneumonia.

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REFERENCES

1. **Arai, S., Y. Gohara, K. Kuwano, and T. Kawashima.** 1992. Antimycoplasmal activities of new quinolones, tetracyclines, and macrolides against *Mycoplasma pneumoniae*. Antimicrob. Agents Chemother. **34**:1322–1324.
2. **Aznar, J., M. C. Caballero, M. C. Lozano, C. De Miguel, J. C. Palomares, and E. J. Perea.** 1985. Activities of new quinolone

derivatives against genital pathogens. Antimicrob. Agents Chemother. **27**:76–78.
3. **Bébéar, C., M. Dupon, H. Renaudin, and B. de Barbeyrac.** 1993. Potential improvements in therapeutic options for mycoplasmal respiratory infections. Clin. Infect. Dis. **17**:S202–S207.
4. **Boerema, J. B. J., D. Bach, C. Jol, and W. Pahlmann.** 1991. Penetration of rufloxacin into human prostatic tissue and fluid. J. Antimicrob. Chemother. **28**:547–554.
5. **Boerema, J. B. J., W. Bischoff, J. Focht, and K. G. Naber.** 1991. An open multicentre study on the efficacy and safety of rufloxacin in patients with chronic bacterial prostatitis. J. Antimicrob. Chemother. **28**:587–597.
6. **Braun, P., J. O. Klein, and E. H. Kass.** 1970. Susceptibility of genital mycoplasmas to antimicrobial agents. Appl. Microbiol. **19**: 62–70.
7. **Cassel, G. H., K. B. Waites, M. S. Pate, K. C. Kanupp, and L. B. Duffy.** 1989. Comparative susceptibility of *Mycoplasma pneumoniae* to erythromycin, ciprofloxacin, and lomefloxacin. Diagn. Microbiol. Infect. Dis. **12**:433–435.
8. **Fallon, R. J., and W. M. Brown.** 1985. In vitro sensitivity of legionellas, meningococci and mycoplasmas to ciprofloxacin and enoxacin. J. Antimicrob. Chemother. **15**:787–789.
9. **Freeman, S., J. Wormuth, and J. Cornett.** 1985. In vitro activity of amifloxacin against *Chlamydia trachomatis* and *Ureaplasma urealyticum*. Eur. J. Clin. Microbiol. **4**:515–516.
10. **Furneri, P. M., M. Bazzano, L. Campo, M. Cesana, and G. Tempera.** 1994. In vitro activity of rufloxacin against Listeria monocytogenes, Legionella pneumophila and Chlamydia trachomatis. Chemotherapy (Basel) **40**:104–108.
11. **Gasser, T. C., S. C. Ebert, P. H. Graversen, and P. O. Madsen.** 1987. Ciprofloxacin pharmacokinetics in patients with normal and impaired renal function. Antimicrob. Agents Chemother. **31**:709–712.
12. **Gollapudi, S. V., S. K. Chuah, T. Harvey, H. D. Thadepalli, and H. Thadepalli.** 1993. In vivo effects of rufloxacin and ciprofloxacin on T-cell subsets and tumor necrosis factor production in mice infected with *Bacteroides fragilis*. Antimicrob. Agents Chemother. **37**:1711–1712.
13. **Hayes, M. M., H.-H. Foo, H. Kotani, D. J. Wear, S.-C. Lo.** 1993. In vitro antibiotic susceptibility testing of different strains of *Mycoplasma fermentans* isolated from a variety of sources. Antimicrob. Agents Chemother. **37**:2500–2503.
14. **Imbimbo, B. P., G. P. Broccali, M. Cesana, F. Crema, and G. Parrinello Attardo.** 1991. Inter- and intrasubject variabilities in the pharmacokinetics of rufloxacin after single oral administration to healthy volunteers. Antimicrob. Agents Chemother. **35**:390–393.
15. **Jalil, N., C. Gilchrist, and D. Taylor-Robinson.** 1989. Factors influencing the in-vitro sensitivity of *Ureaplasma urealyticum* to tetracyclines. J. Antimicrob. Chemother. **23**:341–345.
16. **Kenny, G. E., and F. D. Cartwright.** 1991. Susceptibility of *Mycoplasma pneumoniae* to several new quinolones, tetracycline, and erythromycin. Antimicrob. Agents Chemother. **35**:587–589.
17. **Kenny, G. E., and F. D. Cartwright.** 1993. Susceptibilities of *Mycoplasma hominis*, *Mycoplasma pneumoniae*, and *Ureaplasma urealyticum* to a new quinolone, OPC 17116. Antimicrob. Agents Chemother. **37**:1726–1727.
18. **Kenny, G. E., T. M. Hooton, M. C. Roberts, F. D. Cartwright, and J. Hoyt.** 1989. Susceptibilities of genital mycoplasmas to the newer quinolones as determined by the agar dilution method. Antimicrob. Agents Chemother. **33**:103–107.
19. **Kisicki, J. C., R. S. Griess, C. L. Ott, G. M. Cohen, R. J. McCormack, W. M. Troetel, and B. P. Imbimbo.** 1992. Multiple-dose pharmacokinetics and safety of rufloxacin in normal volunteers. Antimicrob. Agents Chemother. **36**:1296–1301.
20. **Klietmann, W., M. Cesana, R. K. Rondel, and J. Focht.** 1993. Double-blind, comparative study of rufloxacin once daily versus amoxicillin three times a day in treatment of outpatients with exacerbations of chronic bronchitis. Antimicrob. Agents Chemother. **37**:2298–2306.
21. **Krausse, R., and U. Ullmann.** 1988. Comparative in vitro activity of fleroxacin (RO-23-6240) against *Ureaplasma urealyticum* and *Mycoplasma hominis*. Eur. J. Clin. Microbiol. Infect. Dis. **7**:67–69.
22. **Liebowitz, L. D., J. Saunders, G. Fehler, R. C. Ballard, and H. J. Koornhof.** 1986. In vitro activity of A-56619 (difloxacin), A-52620, and other new quinolone antimicrobial agents against genital pathogens. Antimicrob. Agents Chemother. **30**:948–950.
23. **Mattina, R., C. E. Cocuzza, M. Cesana, and G. Bonfiglio.** 1991. In vitro activity of a new quinolone, rufloxacin, against nosocomial isolates. Chemotherapy (Basel) **37**:260–269.
24. **Mattina, R., C. E. Cocuzza, M. Cesana, and the Italian Multicentre UTI-Rufloxacin Group.** 1993. Rufloxacin once daily versus ofloxacin twice daily for treatment of complicated cystitis and upper urinary tract infections. Infection **21**:106–111.
25. **Nakamura, S., A. Ninani, K. Nakata, N. Kurobe, K. Kouno, Y. Sakaguchi, S. Kashimoto, H. Yoshida, T. Kojima, T. Ohue, K. Fujimoto, M. Nakamura, M. Hashimoto, and M. Shimizu.** 1989. In vitro and in vivo antibacterial activities of AT-4140, a new broad spectrum quinolone. Antimicrob. Agents Chemother. **28**:1167–1173.
26. **National Committee for Clinical Laboratory Standards.** 1991. Performance standards for antimicrobial susceptibility testing. Third information supplement. NCCLS document M100-S3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
27. **Osada, Y., and H. Ogawa.** 1983. Antimycoplasmal activity of ofloxacin (DL-8280). Antimicrob. Agents Chemother. **23**:509–511.
28. **Phillips, I., F. Baquero, T. Bergan, J. Degener, G. C. Schito, and B. Wiedemann.** 1993. ESGAB breakpoint determination: rufloxacin. Eur. J. Clin. Microbiol. Infect. Dis. **12**:574–575.
29. **Senterfit, L. B.** 1983. Antibiotic susceptibility testing of mycoplasmas, p. 397–401. *In* S. Razin and J. G. Tully (ed.), Methods in mycoplasmology, vol. 1. Academic Press, Inc., New York.
30. **Shepard, M. C.** 1983. Culture media for ureaplasmas, p. 137–146. *In* S. Razin and J. G. Tully (ed.), Methods in mycoplasmology, vol. 1. Academic Press, Inc., New York.
31. **Taylor-Robinson, D., and P. M. Furr.** 1986. Clinical antibiotic resistance of *Ureaplasma urealyticum*. Pediatr. Infect. Dis. **5**:S335–S337.
32. **Tully, J. G., D. L. Rose, R. F. Whitcomb, and R. P. Wenzel.** 1979. Enhanced isolation of *Mycoplasma pneumoniae* from throat washing with a newly modified culture medium. J. Infect. Dis. **139**:478–482.
33. **Waites, K. B., G. H. Cassel, K. C. Canupp, and P. B. Fernandes.** 1988. In vitro susceptibilities of mycoplasmas and ureaplasmas to new macrolides and aryl-fluoroquinolones. Antimicrob. Agents Chemother. **32**:1500–1502.
34. **Waites, K. B., L. B. Duffy, T. Schmid, D. Crabb, M. S. Pate, and G. H. Cassell.** 1991. In vitro susceptibilities of *Mycoplasma pneumoniae* and *Ureaplasma urealyticum* to sparfloxacin and PD 177391. Antimicrob. Agents Chemother. **35**:1181–1185.
35. **Wise, R., J. M. Andrews, B. P. Imbimbo, and D. Honeybourne.** 1993. The penetration of rufloxacin into sites of potential infection in the respiratory tract. J. Antimicrob. Chemother. **32**:861–866.
36. **Wise, R., J. M. Andrews, R. Matthews, and M. Wolstenholme.** 1992. The in-vitro activity of two new quinolones: rufloxacin and MF 961. J. Antimicrob. Chemother. **29**:649–660.
37. **Wise, R., J. Johnson, N. O'Sullivan, J. M. Andrews, and B. P. Imbimbo.** 1991. Pharmacokinetics and tissue penetration of rufloxacin, a long acting quinolone antimicrobial agent. J. Antimicrob. Chemother. **28**:905–909.