ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1992, p. 1601–1605
0066-4804/92/081601-05$02.00/0

Vol. 36, No. 8

Quinolone Accumulation in *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*

C. McCAFFREY, A. BERTASSO, J. PACE, AND N. H. GEORGOPAPADAKOU*

Roche Research Center, Nutley, New Jersey 07110

Received 8 January 1992/Accepted 11 May 1992

The accumulation of quinolones by *Escherichia coli* JF568, *Pseudomonas aeruginosa* PAO1, and *Staphylococcus aureus* ATCC 29213 was measured by a modified fluorometric assay (J. S. Chapman and N. H. Georgopapadakou, Antimicrob. Agents Chemother. 33:27–29, 1989). The quinolones examined were fleroxacin, pefloxacin, norfloxacin, difloxacin, A56620, ciprofloxacin, ofloxacin, and Ro 09-1168. In all three organisms, uptake was complete in less than 5 min and was proportional to extracellular quinolone concentrations between 2 and 50 μg/ml, which is consistent with simple diffusion. Washing cells with quinolone-free buffer decreased accumulation by up to 70% in *E. coli* and *P. aeruginosa* but not in *S. aureus*. Similarly, incubation with the uncouplers 2,4-dinitrophenol and carbonyl cyanide *m*-chlorophenylhydrazone increased accumulation up to fourfold in *E. coli* and *P. aeruginosa*, though not in *S. aureus*, suggesting endogenous, energy-dependent efflux. High quinolone hydrophobicity was generally associated with decreased accumulation in *E. coli* and *P. aeruginosa* (except in the case of pefloxacin) but was associated with increased accumulation in *S. aureus* (except in the case of difloxacin). Ciprofloxacin had the highest accumulation in *E. coli* and *P. aeruginosa*, while pefloxacin had the highest accumulation in *S. aureus*.

Fluoroquinolones are synthetic antibacterial agents with marked bactericidal activities, broad antibacterial spectra, and favorable pharmacokinetics after oral or parenteral administration (25, 36, 44, 48). Their molecular target is DNA gyrase, a unique and essential bacterial enzyme that catalyzes the negative supercoiling (unwinding) of double-stranded DNA and the decatenation of chromosomes after replication (15, 17–19, 23, 24). Quinolones thus interfere with a variety of processes involving DNA, such as replication, chromosomal segregation, transcription, and recombination.

The ability of quinolones to enter bacterial cells is a factor contributing to their antibacterial potency (4, 41). Both the outer and cytoplasmic membranes are involved; alterations in either or both are associated with decreased quinolone accumulation and low-level resistance (2, 5–7, 10, 43, 47). In enterobacteria, quinolones diffuse across the outer membrane through the porin channels (13, 21, 26, 27, 37) and the phospholipid layer after disrupting the lipopolysaccharide (LPS) (8, 20). In *Pseudomonas aeruginosa*, they diffuse through the D2 protein in the outer membrane and probably through the phospholipid bilayer similarly to enterobacteria (12, 16, 22, 31, 33, 49). The cytoplasmic membrane is less of a barrier for quinolones, which enter by simple diffusion (2, 5, 26). However, quinolone accumulation is reduced by an active efflux system which is especially prominent in quinolone-resistant strains (11, 29, 51). Nevertheless, permeability plays a secondary role in quinolone susceptibility and resistance, in sharp contrast to the situation with β-lactam antibiotics (37).

In this study, we have examined the accumulation of several quinolones in *Escherichia coli*, *P. aeruginosa*, and *Staphylococcus aureus* by using a modification of a previously described fluorometric assay (9). The three organisms were chosen as major gram-negative and gram-positive human pathogens; the latter two have recently acquired additional significance because of the rapid emergence of quinolone-resistant strains (47).

(This work was presented in part at the 91st General Meeting of the American Society for Microbiology [40].)

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* K-12 strains JF568 (39) and TE18 (38) were gifts of J. Foulds of the National Institutes of Health and S. Normark of Washington University, respectively. The standard antibiotic susceptibility strains *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. aureus* ATCC 29213 were purchased from the American Type Culture Collection (Rockville, Md.). *P. aeruginosa* PAO1 was from the Roche culture collection. All bacterial cultures were grown at 37°C in Luria broth.

Quinolones. Fleroxacin (Ro 23-6240) and Ro 09-1168 were obtained from Roche Laboratories (Nutley, N. J.). Norfloxacin was from Merck Sharp & Dohme Research Laboratories (Rahway, N. J.); pefloxacin was from Rhone-Poulenc Pharmaceuticals (Monmouth Junction, N.J.); difloxacin (A56619) and A56620 were from Abbott Laboratories (Chicago, Ill.); ciprofloxacin was from Miles Inc., Pharmaceuticals Division (West Haven, Conn.); sparfloxacin was from Parke-Davis (Ann Arbor, Mich.); and ofloxacin was from Ortho Pharmaceutical Corp. (Raritan, N.J.).

Other chemicals. 2,4-Dinitrophenol (DNP) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) were obtained from Sigma Chemical Co. (St. Louis, Mo.); ^3H_2O (18 μCi/mmol) and [^{14}C]inulin (9 mCi/mmol) were from Amersham Corp. (Arlington Heights, Ill.); and Aquasol was from New England Nuclear Corp. (Boston, Mass.). All other reagents (analytical grade) were from Fisher Scientific Co. (Pittsburgh, Pa.). Culture media were from Difco Laboratories (Detroit, Mich.).

Quinolone hydrophobicity. The hydrophobicities of the quinolones were determined by their partitioning between *n*-octanol and 0.1 M sodium phosphate buffer, pH 7.2 (8, 20).

* Corresponding author.

TABLE 1. Physical properties of quinolones used

Quinolone	Mol wt	Hydrophobicity (P_{app})[a]	λ_{ex}[b] (nm)	λ_{em}[b] (nm)
Fleroxacin	359	0.123	282	442
Pefloxacin	322	1.360	277	442
Norfloxacin	308	0.046	281	440
Difloxacin	399	4.353	280	452
A56620	387	0.135	281	459
Ciprofloxacin	332	0.032	275	448
Ofloxacin	337	0.376	292	496
Ro 09-1168	348	0.143	297	509

[a] P_{app}, partition coefficient in 1-octanol–0.1 M sodium phosphate, pH 7.2 (8, 20).
[b] λ_{ex} and λ_{em}, excitation and emission maxima, respectively, determined in 0.1 M glycine-HCl, pH 3.0.

Quinolone concentrations were determined by quinolone absorbance values at the excitation maximum (Table 1).

Uptake studies. Quinolone accumulation in bacterial cells was assayed by the fluorometric method of Chapman and Georgopapadakou (9), with the following modifications. After incubation with quinolone, cells were pelleted in a water-cooled Savant centrifuge (1 min at 5,000 rpm) without prior addition of 1.5 ml of buffer. They were then washed with 2 ml of quinolone-free buffer at 4°C instead of at room temperature. These modifications increased cellular retention of quinolones by bacteria (29, 31a, 35). Where indicated, cells were treated with 2.0 mM DNP or 0.25 mM CCCP after 10 min of incubation with quinolone. Incubations were performed at least in duplicate (error, ≤10%), and error between repeat experiments was <20%.

Determination of intracellular volume. Intracellular volume was determined from the distribution of water and inulin in the cell pellet (1, 45). Inulin cannot penetrate the outer membrane because of its size and thus serves as a convenient extracellular marker. Cells were grown as for quinolone uptake and suspended in 50 mM sodium phosphate buffer (pH 7.0) to an optical density at 660 nm (OD_{660}) of 20. Aliquots (0.5 ml) were removed and incubated at 37°C for 10 min, ^3H_2O and [^{14}C]inulin were added (final concentrations, 10 and 2 μCi/ml, respectively), and the incubation was continued for another 10 min. The cell suspensions were centrifuged at 5,000 rpm (Savant centrifuge) for 1 min, the supernatant was removed carefully, and the pellet was resuspended in 100 μl of buffer. Aliquots (50 μl) of the supernatant and pellet were removed and counted. Intracellular volume was calculated by the following equation (1, 45): intracellular volume = $[1 - ([^{14}C/^3H \text{ ratio}]_{pellet}/[^{14}C/^3H \text{ ratio}]_{supernatant})] \times 100$.

RESULTS AND DISCUSSION

Physicochemical properties of quinolones. The physicochemical properties of the quinolones used in this study are shown in Table 1. Molecular masses ranged from 300 to 400 Da, and hydrophobicities ranged from 0.046 to 4.353. Fluorescence spectra were similar, with excitation maxima at 275 to 297 nm and emission maxima at 440 to 509 nm. The 5-amino quinolone sparfloxacin, while structurally similar to ciprofloxacin, showed markedly reduced fluorescence (see also reference 42) and was dropped from further studies.

Cell parameters. In an effort to measure the intracellular concentrations of quinolones, the percentage of wet cell pellet representing intracellular volume was determined under assay conditions for quinolone uptake (Table 2). Different cell parameters (cell number, wet weight, dry weight, and protein content) were also correlated with OD_{660} so that quinolone accumulation values obtained for *E. coli*, *S. aureus*, and *P. aeruginosa* could be directly compared with those reported in the literature.

Quinolone uptake. The concentrations of quinolones accumulated by the three organisms at 37°C over a 30-min time course are shown in Fig. 1. Maximum levels of most quinolones were reached in less than 5 min. Accumulation was proportional to the external quinolone concentration between 2 and 50 μg/ml (data not shown), confirming published reports that uptake occurs by simple diffusion (3, 29, 46). Subsequently, a 10-μg/ml quinolone concentration was used throughout the study. Quinolone accumulation in unwashed cells of the three organisms varied between 150 and 300 ng/mg (dry weight) of cells at the 10-μg/ml extracellular quinolone concentration (Fig. 1). This corresponds to a 5- to 10-fold intracellular accumulation. Quinolone accumulation was significantly lower in washed cells of *E. coli* and *P. aeruginosa*, though it remained relatively unchanged in washed cells of *S. aureus*.

Blocking energy production by the uncouplers DNP and CCCP appeared to increase quinolone accumulation significantly in *E. coli* and *P. aeruginosa* but only slightly in *S. aureus* (Fig. 2). In previous studies we were unable to show any effect of DNP on fleroxacin accumulation by *E. coli* (8). This was probably due to the extensive loss of cell-associated quinolone after the cells were washed at room temperature. In the modified assay, all steps subsequent to quinolone loading were carried out at 4°C. Similar conditions have been recently adopted by other investigators (29, 35). Ofloxacin was more affected than the less hydrophobic fleroxacin, a situation reminiscent of that with tetracyclines (32). Active efflux may thus be largely responsible for the loss of cell-associated quinolone by washing. DNP and CCCP have been reported to increase quinolone accumula-

TABLE 2. Cell parameters in different bacteria at an OD_{660} of 1

Organism	CFU/ml	Concn (mg/ml) of:			Intracellular vol (% wet wt)
		Cells (wet wt)	Cells (dry wt)	Protein	
E. coli					
ATCC 25922	9×10^8	3.0 ± 0.1	0.44 ± 0.02	0.24 ± 0.04	55
JF568	ND[a]	2.5 ± 0.1	0.41 ± 0.01	0.21 ± 0.04	ND
P. aeruginosa PAO1	8×10^8	2.7 ± 0.2	0.42 ± 0.02	0.16 ± 0.01	ND
S. aureus ATCC 29213	5×10^8	1.5 ± 0.1	0.30 ± 0.02	0.18 ± 0.02	52

[a] ND, not determined.

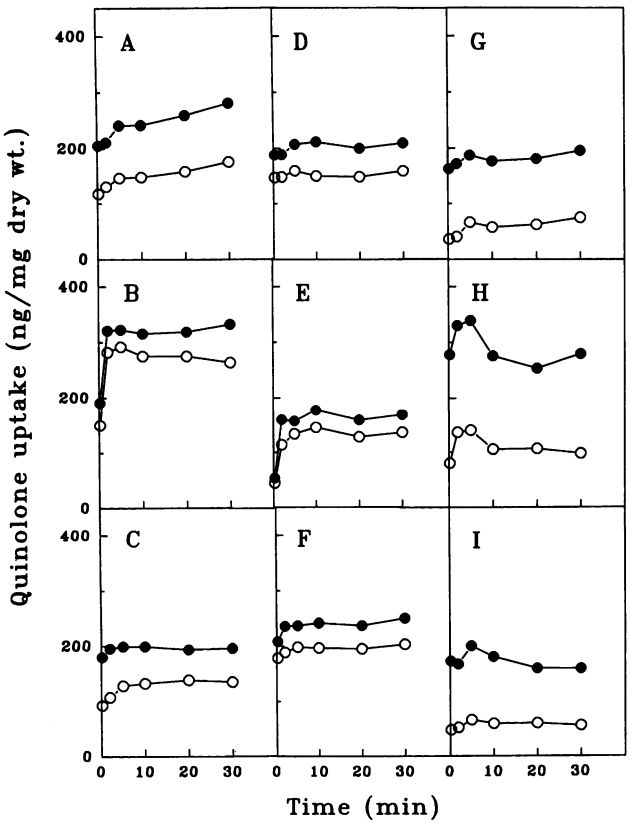

FIG. 1. Quinolone uptake by *E. coli* JF568 (A through C), *S. aureus* ATCC 29213 (D through F), and *P. aeruginosa* PAO1 (G through I). The quinolones used were fleroxacin (A, D, and G), ciprofloxacin (B, E, and H), and ofloxacin (C, F, and I). Open and closed circles, washed and unwashed cells, respectively.

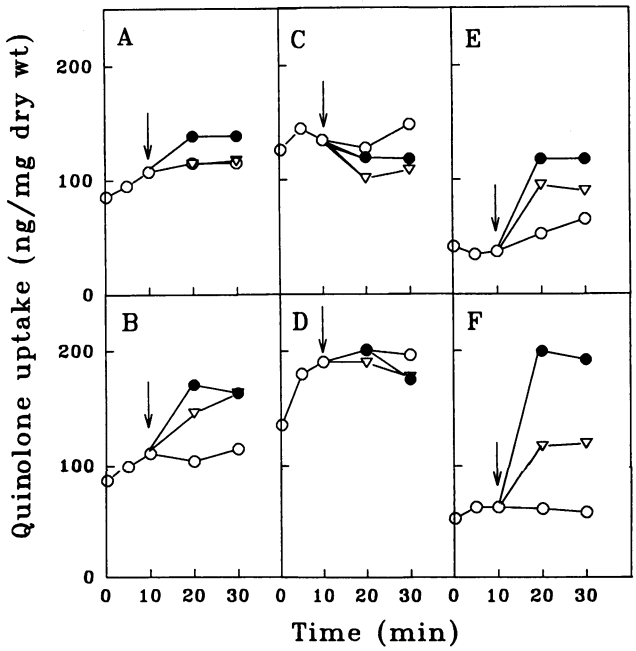

FIG. 2. Quinolone uptake by *E. coli* JF568 (A and B), *S. aureus* ATCC 29213 (C and D), and *P. aeruginosa* PAO1 (E and F). The quinolones used were fleroxacin (A, C, and E) and ofloxacin (B, D, and F). The addition of 0.25 mM CCCP (●) and 2 mM DNP (▽) to control (○) is indicated by arrows.

tion by clinical isolates of *S. aureus* (28, 50, 51). The variability could be due to the different strains and media used, as has been proposed for other species (2). In this context, it is interesting that DNP has been reported to inhibit quinolone uptake by *E. coli* in one study (14) and to stimulate it in another (10). Similarly, *E. coli* MAL300, a strain unable to maintain a transmembrane potential at elevated temperatures (30), showed no increased uptake of fleroxacin or ofloxacin at the restrictive temperature (31a).

Quinolone hydrophobicity was negatively associated with uptake in *E. coli*, especially the smooth strain ATCC 25922, except in the case of pefloxacin (Table 3). The association was less pronounced in the K-12 strains TE18 and JF568, which lack the long-chain O-polysaccharide component of LPS, and in *P. aeruginosa* PAO1. In both *E. coli* and *P. aeruginosa*, norfloxacin and ciprofloxacin showed the highest accumulations. Quinolone hydrophobicity was positively associated with uptake in *S. aureus*, except in the case of difloxacin. Pefloxacin had the highest accumulation in this organism.

Various studies have reported quinolone accumulation per OD unit of cell suspension (3), number of cells (46), milligram (wet weight) of cells (34), milligram (dry weight) of cells (21), and milligram of protein (10). Throughout this article, quinolone accumulation is expressed in nanograms per milligram (dry weight) of cells. However, by using the appropriate conversion factors from Table 2, quinolone accumulation can also be expressed in nanograms per microliter of intracellular volume. Thus, 100 ng of quinolone per mg (dry weight) of *E. coli* ATCC 25922 cells is equivalent to 16 ng/mg (wet weight) of cells or 26 ng/μl of intracellular volume (versus the 10-ng/μl external concentration). All quinolones showed concentrative uptake in *E. coli* (≥40 ng/mg [dry weight] of cells) even after the cells were washed (Table 3).

In conclusion, the present work improves and extends the previously developed fluorometric assay for quinolone uptake to more quinolones and organisms. In the process, it examines the impact of efflux on concentrative uptake. Finally, the surprisingly poor fluorescence of sparfloxacin points to a major limitation of the fluorometric assay.

TABLE 3. Quinolone uptake by bacteria (washed cells) at a 10-μg/ml external concentration after 10 min of incubation

Quinolone	Uptake (ng/mg [dry wt] of cells)					
	E. coli			*P. aeruginosa* PAO1	*S. aureus*	
	ATCC 25922	TE18	JF568		ATCC 29213	ATCC 25923
Fleroxacin	94	103	128	57	142	157
Pefloxacin	145	163	168	95	245	353
Norfloxacin	211	170	226	105	188	ND[a]
Difloxacin	46	111	64	55	68	185
A56620	95	121	138	72	150	ND
Ciprofloxacin	283	202	281	101	145	ND
Ofloxacin	97	147	120	64	194	187
Ro 09-1168	60	89	81	32	134	ND

[a] ND, not determined.

REFERENCES

1. **Bakker, E. P., H. Rottenberg, and S. R. Kaplan.** 1976. An estimation of the light-induced electrochemical potential difference of protons across the membrane of *Halobacterium halobium*. Biochim. Biophys. Acta **440**:557–572.
2. **Bedard, J., S. Chamberland, S. Wong, T. Schollaardt, and L. E. Bryan.** 1989. Contribution of permeability and sensitivity to inhibition of DNA synthesis in determining susceptibilities of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Alcaligenes faecalis* to ciprofloxacin. Antimicrob. Agents Chemother. **33**: 1457–1464.
3. **Bedard, J., S. Wong, and L. E. Bryan.** 1987. Accumulation of enoxacin by *Escherichia coli* and *Bacillus subtilis*. Antimicrob. Agents Chemother. **31**:1348–1354.
4. **Bryan, L. E., and J. Bedard.** 1991. Impermeability to quinolones in gram-positive and gram-negative bacteria. Eur. J. Clin. Microbiol. Infect. Dis. **10**:232–239.
5. **Celesk, R. A., and N. J. Robillard.** 1989. Factors influencing the accumulation of ciprofloxacin in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **33**:1921–1926.
6. **Chamberland, S., A. S. Bayer, T. Schollaardt, S. A. Wong, and L. E. Bryan.** 1989. Characterization of mechanisms of quinolone resistance in *Pseudomonas aeruginosa* strains isolated in vitro and in vivo during experimental endocarditis. Antimicrob. Agents Chemother. **33**:624–634.
7. **Chapman, J. S., A. Bertasso, and N. H. Georgopapadakou.** 1989. Fleroxacin resistance in *Escherichia coli*. Antimicrob. Agents Chemother. **33**:239–241.
8. **Chapman, J. S., and N. H. Georgopapadakou.** 1988. Routes of quinolone permeation in *Escherichia coli*. Antimicrob. Agents Chemother. **32**:438–442.
9. **Chapman, J. S., and N. H. Georgopapadakou.** 1989. Fluorometric assay for fleroxacin uptake by bacterial cells. Antimicrob. Agents Chemother. **33**:27–29.
10. **Cohen, S. P., D. C. Hooper, J. S. Wolfson, K. S. Souza, L. M. McMurry, and S. B. Levy.** 1988. Endogenous active efflux of norfloxacin in susceptible *Escherichia coli*. Antimicrob. Agents Chemother. **32**:1187–1191.
11. **Cohen, S. P., L. M. McMurry, D. C. Hooper, J. S. Wolfson, and S. B. Levy.** 1989. Cross-resistance to fluoroquinolones in multiple-antibiotic-resistant (Mar) *Escherichia coli* selected by tetracycline or chloramphenicol: decreased drug accumulation associated with membrane changes in addition to OmpF reduction. Antimicrob. Agents Chemother. **33**:1318–1325.
12. **Daikos, G. L., V. T. Lolans, and G. G. Jackson.** 1988. Alterations in the outer membrane proteins of *Pseudomonas aeruginosa* associated with selective resistance to quinolones. Antimicrob. Agents Chemother. **32**:785–787.
13. **Dechene, M., H. Leying, and W. Cullmann.** 1990. Role of the outer membrane for quinolone resistance in enterobacteria. Chemotherapy (Basel) **36**:13–23.
14. **Diver, J. M., L. J. V. Piddock, and R. Wise.** 1990. The accumulation of five quinolone antibacterial agents by *Escherichia coli*. J. Antimicrob. Chemother. **25**:319–333.
15. **Drlica, K.** 1984. Biology of bacterial deoxyribonucleic acid topoisomerases. Microbiol. Rev. **48**:273–289.
16. **Fukuda, H., M. Hosaka, K. Hirai, and S. Iyobe.** 1990. New norfloxacin resistance gene in *Pseudomonas aeruginosa* PAO. Antimicrob. Agents Chemother. **34**:1757–1761.
17. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50**:879–910.
18. **Gellert, M., R. Menzel, K. Mizuuchi, M. H. O'Dea, T. Itoh, and D. I. Friedman.** 1982. Regulation of DNA supercoiling in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **47**: 763–767.
19. **Georgopapadakou, N. H., B. A. Dix, P. Angehrn, A. Wick, and G. L. Olson.** 1987. Monocyclic and tricyclic analogs of quinolones: mechanism of action. Antimicrob. Agents Chemother. **31**:614–616.
20. **Hirai, K., H. Aoyama, T. Irikura, S. Iyobe, and S. Mitsuhashi.** 1986. Differences in susceptibility to quinolones of outer membrane mutants of *Salmonella typhimurium* and *Escherichia coli*. Antimicrob. Agents Chemother. **29**:535–538.
21. **Hirai, K., H. Aoyama, S. Suzue, T. Irikura, S. Iyobe, and S. Mitsuhashi.** 1986. Isolation and characterization of norfloxacin-resistant mutants of *Escherichia coli* K-12. Antimicrob. Agents Chemother. **30**:248–253.
22. **Hirai, K., S. Suzue, T. Irikura, S. Iyobe, and S. Mitsuhashi.** 1987. Mutations producing resistance to norfloxacin in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **31**:582–586.
23. **Hooper, D. C., and J. S. Wolfson.** 1989. Mode of action of the quinolone antimicrobial agents: review of recent information. Rev. Infect. Dis. **2**(Suppl. 5):902–910.
24. **Hooper, D. C., and J. S. Wolfson.** 1991. Mode of action of the new quinolones: new data. Eur. J. Clin. Microbiol. Infect. Dis. **10**:223–231.
25. **Hooper, D. C., and J. S. Wolfson.** 1991. Fluoroquinolone antimicrobial agents. N. Engl. J. Med. **324**:384–394.
26. **Hooper, D. C., J. S. Wolfson, K. S. Souza, E. Y. Ng, G. L. McHugh, and M. N. Swartz.** 1989. Mechanisms of quinolone resistance in *Escherichia coli*: characterization of *nfxB* and *cfxB*, two mutant resistance loci decreasing norfloxacin accumulation. Antimicrob. Agents Chemother. **33**:283–290.
27. **Hooper, D. C., J. S. Wolfson, K. S. Souza, C. Tung, G. L. McHugh, and M. N. Swartz.** 1986. Genetic and biochemical characterization of norfloxacin resistance in *Escherichia coli*. Antimicrob. Agents Chemother. **29**:639–644.
28. **Kaatz, G. W., S. M. Seo, and C. A. Ruble.** 1991. Mechanisms of fluoroquinolone resistance in *Staphylococcus aureus*. J. Infect. Dis. **163**:1080–1086.
29. **Lei, Y., K. Sato, and T. Nakae.** 1991. Ofloxacin-resistant *Pseudomonas aeruginosa* mutants with elevated drug extrusion across the inner membrane. Biochem. Biophys. Res. Commun. **178**:1043–1048.
30. **Lieberman, M. A., M. Simon, and J. S. Hong.** 1977. Characterization of *Escherichia coli* mutant incapable of maintaining a transmembrane potential. J. Biol. Chem. **252**:4056–4062.
31. **Masecar, B. L., R. A. Celesk, and N. J. Robillard.** 1990. Analysis of acquired ciprofloxacin resistance in a clinical strain of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **34**:281–286.

31a. **McCaffrey, C., and N. H. Georgopapadakou.** Unpublished results.

32. **McMurry, L. M., D. A. Aronson, and S. B. Levy.** 1983. Susceptible *Escherichia coli* cells can actively excrete tetracyclines. Antimicrob. Agents Chemother. **24**:544–551.
33. **Michéa-Hamzehpour, M., Y. X. Furet, and J.-C. Pechère.** 1991. Role of protein D2 and lipopolysaccharide in diffusion of quinolones through the outer membrane of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **35**:2091–2097.
34. **Moniot-Ville, N., J. Guibert, N. Moreau, J. F. Acar, E. Collatz, and L. Gutmann.** 1991. Mechanisms of quinolone resistance in a clinical isolate of *Escherichia coli* highly resistant to fluoroquinolones but susceptible to nalidixic acid. Antimicrob. Agents Chemother. **35**:519–523.
35. **Mortimer, P. G. S., and L. J. V. Piddock.** 1991. A comparison of methods used for measuring the accumulation of quinolones by Enterobacteriaceae, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. J. Antimicrob. Chemother. **28**:639–653.
36. **Neu, H. C.** 1989. Clinical utility of DNA gyrase inhibitors. Pharmacol. Ther. **41**:207–221.
37. **Nikaido, H.** 1989. Outer membrane barrier as a mechanism of antimicrobial resistance. Antimicrob. Agents Chemother. **33**: 1831–1836.
38. **Nikaido, H., and S. Normark.** 1987. Sensitivity of *Escherichia coli* to various β-lactams is determined by the interplay of outer membrane permeability and degradation by periplasmic β-lactamases: a quantitative predictive treatment. Mol. Microbiol. **1**:29–36.
39. **Nikaido, H., E. Y. Rosenberg, and J. Foulds.** 1983. Porin channels in *Escherichia coli*: studies with β-lactams in intact cells. J. Bacteriol. **153**:232–240.
40. **Pace, J., A. Bertasso, and N. H. Georgopapadakou.** 1991.

Accumulation of tricyclic and bicyclic quinolones in *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, abstr. A-90, p. 16. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991.

41. **Piddock, L. J. V.** 1991. Mechanism of quinolone uptake into bacterial cells. J. Antimicrob. Chemother. **27**:399–403.
42. **Piddock, L. J. V., and M. Zhu.** 1991. Mechanism of action of sparfloxacin against and mechanism of resistance in gram-negative and gram-positive bacteria. Antimicrob. Agents Chemother. **35**:2423–2427.
43. **Robillard, N. J., and A. L. Scarpa.** 1988. Genetic and physiological characterization of ciprofloxacin resistance in *Pseudomonas aeruginosa* PAO. Antimicrob. Agents Chemother. **32**: 535–539.
44. **Siporin, C.** 1989. The evolution of fluorinated quinolones: pharmacology, microbiological activity, clinical uses, and toxicities. Annu. Rev. Microbiol. **43**:601–627.
45. **Stock, J. B., B. Rauch, and S. Roseman.** 1977. Periplasmic space in *Salmonella typhimurium* and *Escherichia coli*. J. Biol. Chem. **252**:7850–7861.
46. **Valisena, S., M. Palumbo, C. Parolin, G. Palu, and G. A. Meloni.** 1990. Relevance of ionic effects on norfloxacin uptake by *Escherichia coli*. Biochem. Pharmacol. **40**:431–436.
47. **Wolfson, J. S., and D. C. Hooper.** 1989. Bacterial resistance to quinolones: mechanisms and clinical importance. Rev. Infect. Dis. **11**(Suppl.):960–968.
48. **Wolfson, J. S., and D. C. Hooper.** 1989. Fluoroquinolone antimicrobial agents. Clin. Microbiol. Rev. **2**:378–424.
49. **Yamano, Y., T. Nishikawa, and Y. Komatsu.** 1990. Outer-membrane proteins responsible for the penetration of β-lactams and quinolones in *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **26**:175–184.
50. **Yoshida, H., M. Bogaki, S. Nakamura, K. Ubukata, and M. Konno.** 1990. Nucleotide sequence and characterization of the *Staphylococcus aureus norA* gene, which confers resistance to quinolones. J. Bacteriol. **172**:6942–6949.
51. **Yoshida, S., T. Kojima, M. Inoue, and S. Mitsuhashi.** 1991. Uptake of sparfloxacin and norfloxacin by clinical isolates of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **35**: 368–370.