ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1988, p. 535–539
0066-4804/88/040535-05$02.00/0
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Vol. 32, No. 4

Genetic and Physiological Characterization of Ciprofloxacin Resistance in *Pseudomonas aeruginosa* PAO

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Received 31 August 1987/Accepted 21 December 1987

Spontaneous ciprofloxacin-resistant mutants of *Pseudomonas aeruginosa* PAO2 were isolated on ML agar containing 0.5 μg of ciprofloxacin per ml (2 times the MIC). The mutants were 8- to 64-fold more resistant to ciprofloxacin and showed complete cross resistance to nalidixic acid, ofloxacin, enoxacin, and norfloxacin. Two chromosomal resistance genes, *cfxA* and *cfxB*, were mapped between *eda-9001* and *phe-2* and near *pyrB52* distal to *proC130*, respectively. The *cfxB* mutation was identical to a *nalB* mutation and conferred cross resistance to novobiocin, tetracycline, carbenicillin, and chloramphenicol, suggesting that there is an effect on permeability. DNA gyrase A and B subunits were purified from strain PAO2 (wild type), PAO236 *nalA2*, PAO4704 *cfxA2*, and PAO4700 *cfxA1 cfxB1*. Inhibition of gyrase-mediated DNA supercoiling by ciprofloxacin or nalidixic acid was greatly reduced in preparations derived from each of the mutants. Inhibition studies on reconstituted heterologous gyrase subunits showed that decreased inhibition was dependent on the mutant gyrase A subunit. We conclude that ciprofloxacin resistance in *P. aeruginosa* PAO2 can occur by mutation in the *nalB* gene or the gene for DNA gyrase A (formerly *nalA*).

Ciprofloxacin possesses good in vitro activity and is therapeutically effective against *Pseudomonas aeruginosa* (2, 6, 7, 15). In *P. aeruginosa* and *Escherichia coli*, the potent antibacterial activity of ciprofloxacin correlates with its ability to inhibit the enzyme DNA gyrase (11, 12). DNA gyrase is composed of two dimeric subunits. The A subunit, which is susceptible to quinolone inhibition, is responsible for the breakage and reunion of DNA, while the B subunit is the site of ATP hydrolysis (4, 28). DNA gyrase, using energy supplied by ATP, introduces negative superhelical twists into covalently closed circular DNA molecules (4, 9). In the absence of ATP the negative superhelical twists are removed, thus relaxing the DNA. The action of this essential enzyme is required for DNA replication and is involved in recombination, repair, and transcription (9).

Hooper et al. (11), working with *E. coli*, reported on two ciprofloxacin resistance mutations designated *cfxA* and *cfxB*. The *cfxA* mutation was mapped to a location on the *E. coli* chromosome which is consistent with a *gyrA* mutation. However, DNA gyrase from this mutant was not assayed for in vitro resistance to ciprofloxacin. The *cfxB* mutation was determined to be an allele of *marA* and appeared to alter outer membrane permeability. Sanders et al. (25) have documented the occurrence of ciprofloxacin resistance in vitro in *P. aeruginosa*, but the basis for the resistance was not investigated. We investigated ciprofloxacin resistance in *P. aeruginosa* PAO2 to determine the following: (i) the chromosomal location of the resistance mutations, (ii) the mechanism(s) by which resistance is mediated, and (iii) the relative contribution of each class of mutation to resistance. We identified two classes of resistance mutations, *cfxA* and *cfxB*. The *cfxA* mutation alters the A subunit of DNA gyrase, while the *cfxB* mutation appears to alter permeability. This is the first report in which a specific ciprofloxacin resistance mutation has been shown to result in an alteration in DNA gyrase.

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MATERIALS AND METHODS

Bacterial strains and phages. The *P. aeruginosa* PAO strains used in this study are listed in Table 1. The generalized transducing phage F116L was obtained from Paul Phibbs, East Carolina School of Medicine, Greenville, N.C.

Growth media. ML broth has been described previously (19). For ML agar plates, 1.5% agar (Difco Laboratories, Detroit, Mich.) was added. Mueller-Hinton broth (Difco) was supplemented with $MgCl_2 \cdot 6H_2O$ and $CaCl_2 \cdot 2H_2O$ to final concentrations of 25 and 50 mg/liter, respectively. Basal salts medium has been described previously (24). Glucose was added to a final concentration of 20 mM. Required amino acids were added to a final concentration of 1 mM.

Antibiotics. The following antibiotics were used in this study and were supplied by the indicated sources: chloramphenicol, novobiocin, and tetracycline (Sigma Chemical Co., St. Louis, Mo.), ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.), nalidixic acid (Sterling Winthrop, Renssalaer, N.Y.), norfloxacin (Merck & Co., Inc., Rahway, N.J.), ofloxacin (Ortho Diagnostics, Inc., Raritan, N.J.), enoxacin (Warner-Lambert Co., Ann Arbor, Mich.), and carbenicillin (Beecham Laboratories, Bristol, Tenn.).

Isolation of ciprofloxacin-resistant mutants. Cells of *P. aeruginosa* PAO2 were grown overnight in 5 ml of ML broth at 32°C with aeration. The culture was diluted 1:100 in 50 ml of fresh ML broth and incubated at 35°C overnight with aeration. The cells were centrifuged at 10,000 rpm in a Sorval SS34 rotor and suspended in 2 ml of ML broth. Dilutions of the cells were plated on ML agar and incubated at 35°C overnight to obtain the number of CFU per milliliter. ML agar plates containing 0.5 μg of ciprofloxacin per ml were spread with several 0.1-ml portions of cells. The selection plates were incubated at 35°C for 1 to 2 days.

Determination of MICs. Standard microdilution techniques were used with cation-supplemented Mueller-Hinton broth as the growth medium for all MIC determinations except novobiocin (20). Novobiocin MICs were determined by broth macrodilution with basal salts medium supplemented

TABLE 1. *P. aeruginosa* strains used in this study

Strain	Genotype	Reference or derivation
PAO2	*ser-3*	Holloway collection[a]
PAO4	*pyrB52 arg-47*	Holloway collection
PAO660	*phe-2*	Holloway collection
PAO6005	*proC130 nalB8*	Rella and Haas (23)
PAO969	*proC130*	Rella and Haas (23)
PAO236	*ilv-226 his-4 lys-12 met-28 trp-6 proA82-nalA2*	Phibbs collection[b]
PAO1838	*eda-9001 met-9020*	Phibbs collection
PAO4700	*cfxA1 cfxB1 ser-3*	This study
PAO4701	*cfxA2 ser-3*	This study
PAO4702	*cfxA3 ser-3*	This study
PAO4703	*cfxA4 ser-3*	This study
PAO4731	*cfxA1 met-9020*	Hex$^+$ F116L transductant PAO4700 × PAO1838
PAO4727	*cfxB1 arg-47*	Pyr $^+$ F116L transductant PAO4700 × PAO4

[a] Bruce Holloway, Department of Genetics, Monash University, Clyton, Victoria, Australia.
[b] Paul Phibbs, School of Medicine, East Carolina University, Greenville, N.C.

with glucose and any required amino acids. Nalidixic acid MICs were confirmed by agar dilution when precipitation at high drug concentrations was encountered.

Genetic mapping. The generalized transducing phage F116L (13) was propagated on donor bacterial strains as reported previously (3, 8); however, ML agar was used as the enriched medium. The phage lysates were stored at 4°C over chloroform with no detectable loss of viable phage. Transductions were performed by previously published methods (22, 24) by using a multiplicity of infection of approximately 1.0.

Isolation of DNA gyrase. Cells were grown in 10 liters of ML broth in a fermentor (MF-114; New Brunswick Scientific Co., Inc., Edison, N.J.). The cells were harvested when the optical density measured at 600 nm reached 1.0. Approximately 30 g of cells (two batches) was washed and suspended in 10% sucrose–50 mM Tris hydrochloride (pH 7.5). The suspension was frozen in liquid nitrogen and stored at −70°C. Cell lysis and streptomycin sulfate precipitation were performed as described previously (26). Solid ammonium sulfate was added to the lysate to give a final concentration of 0.33 μg/ml. The lysate was stirred on ice for 1 h and centrifuged at 10,000 × *g* for 20 min at 4°C. The DNA gyrase A and B proteins contained in the pellet were isolated by the novobiocin-Sepharose procedure of Staudenbauer and Orr (27). DNA gyrase subunits from strain PAO2 were designated A_sB_s, while DNA gyrase subunits from ciprofloxacin-resistant or nalidixic acid-resistant mutants were designated A_rB_r. The amount of enzyme recovered was between 0.2 and 0.5 mg. Preparations of the B subunit were approximately 80% pure, as judged by protein gels, while preparations of the A subunit were less pure. Approximately 15,000 units, as defined below, were recovered in each preparation.

DNA supercoiling assay and IC_{50} determinations. The DNA supercoiling assay of Mizuuchi et al. (17, 18) was used. A unit of activity was defined as the amount of enzyme which converts 50% of a 0.4-μg portion of relaxed pBR322 plasmid DNA to the supercoiled form in 60 min at 25°C. Approximately 3 units of DNA gyrases A and B were used in each reaction. Ciprofloxacin or nalidixic acid was added to the reactions from 10× aqueous stock solutions. The IC_{50} was defined as the concentration of drug which inhibited approximately half of the supercoiling activity. This value was determined by scanning the most highly supercoiled plasmid band on gel negatives with a densitometer (Joyce Loebl).

RESULTS

Mapping of mutations conferring resistance to ciprofloxacin. Spontaneous ciprofloxacin-resistant mutants of *P. aeruginosa* PAO2 were obtained at a frequency of 1.5×10^{-7} on ML agar containing 0.5 μg of ciprofloxacin per ml. Several mutants were purified twice on the same medium. All of the mutants retained the PAO2 *ser-3* marker. Four of the mutants (PAO4700, PAO4701, PAO4702, and PAO4703) were selected for further study. F116L-mediated transductions were performed to locate the ciprofloxacin-resistance mutations on the PAO chromosome. The results are shown in Table 2. Four ciprofloxacin resistance mutations (*cfxA1* in PAO4700, *cfxA2* in PAO4701, *cfxA3* in PAO4702, and *cfxA4* in PAO4703) were cotransducible with *eda-9001* (50 to 58% linkage) and *phe-2* (76 to 80% linkage). Based on the location of *eda-9001* and *phe-2* (5), the marker order must be *phe-2 cfxA eda-9001*. This places *cfxA* in the *nalA* region at 39 min on the recalibrated PAO linkage map of Holloway et al. (10a). PAO4700 also contained a *cfxB* mutation which was mapped by transduction with phage F116L. *cfxB* was cotransducible with *pyrB52* (32% linkage) and *proC130* (4.7% linkage). Based on the locations of *pyrB52* and *proC130* (23), the marker order must be *proC130 pyrB52 cfxB1*, thus placing *cfxB* in the *nalB* region at 20 min on the genetic map.

Susceptibility to quinolones and other antibiotics. The mutants exhibited an 8- to 64-fold decrease in susceptibility to ciprofloxacin, with PAO4700 being the least susceptible (Table 3). All of the mutants showed complete cross resistance to the other quinolones tested. PAO4700 was unique, as it not only was the most resistant to quinolones but it also

TABLE 2. Cotransduction of ciprofloxacin resistance mutations

Donor (recipient) strain	Selected marker	Unselected marker	No. of cotransductants/ total (%)[a]
PAO4700			
PAO1838	*eda-9001*$^+$	Cip^{r}[b]	129/238 (54)
PAO660	*phe-2*$^+$	Cip^r	80/100 (80)
PAO4	*pyrB52*$^+$	Nal^{r}[c]	32/100 (32)
PAO969	*proC130*$^+$	Nal^r	7/150 (5)
PAO4701			
PAO1838	*eda-9001*$^+$	Cip^r	100/200 (50)
PAO660	*phe-2*$^+$	Cip^r	76/100 (76)
PAO4	*pyrB52*$^+$	Nal^r	0/100 (0)
PAO4702			
PAO1838	*eda-9001*$^+$	Cip^r	134/232 (58)
PAO660	*phe-2*$^+$	Cip^r	76/100 (76)
PAO4	*pyrB52*$^+$	Nal^r	0/100 (0)
PAO4703			
PAO1838	*eda-9001*$^+$	Cip^r	57/100 (57)
PAO660	*phe-2*$^+$	Cip^r	76/100 (76)
PAO4	*pyrB52*$^+$	Nal^r	0/100 (0)

[a] The fractions represent the number of transductants carrying the unselected marker over the total number of transductants tested. The values in parentheses are percent cotransduction, obtained by multiplying the fraction by 100.
[b] ML agar containing 0.5 μg of ciprofloxacin per ml was used to score for inheritance of the *cfxA* marker.
[c] ML agar containing 500 μg of nalidixic acid per ml was used to score for inheritance of the *cfxB* marker.

TABLE 3. Susceptibility of ciprofloxacin-resistant mutants of *P. aeruginosa* PAO2 to quinolones

Strain or mutant	MIC (μg/ml)				
	Ciprofloxacin	Norfloxacin	Ofloxacin	Enoxacin	Nalidixic acid
PAO2 (wild type)	0.25	1.0	2.0	1.0	50
PAO4700 (*cfxA1 cfxB1*)	16.0	32.0	>32.0	32.0	>3,200
PAO4701 (*cfxA2*)	2.0	8.0	8.0	8.0	3,200
PAO4702 (*cfxA3*)	4.0	16.0	16.0	16.0	3,200
PAO4703 (*cfxA4*)	4.0	16.0	16.0	16.0	3,200
PAO236 (*nalA2*)	16.0	32.0	32.0	32.0	3,200
PAO6005 (*nalB8*)	1.0	2.0	8.0	4.0	400
PAO4731 (*cfxA1*)	2.0	4.0	8.0	8.0	3,200
PAO4727 (*cfxB1*)	2.0	4.0	8.0	4.0	400

exhibited broad cross resistance to nonquinolone antibiotics (Table 4). PAO4700 was less susceptible to novobiocin, carbenicillin, chloramphenicol, and tetracycline. To determine whether the *cfxB1* mutation in PAO4700 was responsible for the cross resistance to nonquinolone antibiotics, the *cfxB1* and *cfxA1* mutations harbored by PAO4700 were separated by transduction. PAO4727 *cfxB1* and PAO4731 *cfxA1* were compared with PAO2 (wild type), PAO4700 *cfxA1 cfxB1*, PAO6005 *nalB8*, PAO4701 *cfxA2*, and PAO4703 *cfxA3* for cross resistance to the nonquinolone antibiotics listed above. The *cfxB1*, but not the *cfxA1*, mutation conferred decreased susceptibility to novobiocin, tetracycline, carbenicillin, and chloramphenicol (Table 4). This pattern of cross resistance is similar to that of PAO6005, which was previously shown to exhibit cross resistance to novobiocin and β-lactams (23). These results, together with the close proximity of *cfxB1* and *nalB8*, suggest that both mutations occur in the same gene or operon. This type of broad cross resistance encompassing several classes of antibiotics is suggestive of an alteration in permeability. In light of these findings, it is not surprising that we have observed an alteration in the outer membrane protein profile of this mutant (unpublished data). The relative contribution of the *cfxA1* and *cfxB1* mutations to quinolone resistance also was investigated. The MICs of PAO4731 *cfxA1*, PAO4727 *cfxB1*, and PAO6005 *nalB8* for various quinolones are included in Table 3. The *cfxA1* mutation produced a low-level resistance pattern that was identical to that of *cfxA2*. The *cfxB1* resistance pattern was very similar to that of *cfxA1* with respect to the newer quinolones; however, the *cfxB1* mutant exhibited an 8-fold increase in resistance to nalidixic acid, while the *cfxA* mutants exhibited a 64-fold increase in resistance to nalidixic acid.

Effect of *cfxA* and *nalA* mutations on DNA gyrase. To determine whether *cfxA* mutations occur in the *nalA* gene and whether this gene codes for the enzyme DNA gyrase, DNA gyrase A and B subunits were purified from PAO2, PAO4700 *cfxA1 cfxB1*, PAO4701 *cfxA2*, and PAO236 *nalA2*. The IC_{50}s of ciprofloxacin and nalidixic acid were determined for each enzyme. The results are shown in Table 5. The IC_{50}s for wild-type DNA gyrase from PAO2 were 0.25 and 25 μg/ml for ciprofloxacin and nalidixic acid, respectively. DNA gyrase from PAO236 *nalA2*, PAO4700 *cfxA1 cfxB1*, and PAO4731 *cfxA2* was significantly more resistant to supercoiling inhibition by ciprofloxacin and nalidixic acid. The IC_{50}s for PAO4700, PAO4701, and PAO236 were at least 32-fold greater for ciprofloxacin and 64-fold greater for nalidixic acid. These results provide evidence that *cfxA* and *nalA* mutations occur in the same gene and that these mutations affect the enzyme DNA gyrase. To confirm that this genetic locus codes for the A subunit of DNA gyrase, IC_{50} assays were carried out on heterologous DNA gyrase preparations derived from PAO2 (A_sB_s) and PAO236 (A_rB_r). The results presented in Table 6 indicate that the sensitivity of A_rB_s gyrase to ciprofloxacin and nalidixic acid was identical to that of A_rB_r, while the sensitivity of A_sB_r to ciprofloxacin and nalidixic acid was the same as that of A_sB_s. This correlation also holds for heterologous subunits from PAO2, PAO4700, and PAO4704 (data not shown). We conclude from these results that *cfxA* and *nalA* mutations occur in the gene for DNA gyrase A. Similar results were obtained recently for a *nalA* mutation by Inoue et al. (12).

DISCUSSION

We isolated several ciprofloxacin-resistant mutants of *P. aeruginosa* PAO2. All of the mutants were reidentified by the *ser-3* marker harbored by PAO2. With the exception of *cfxB1*, the mutations mapped in the *nalA* region at 39 min on the recalibrated map (10a). Our F116L linkage values for *cfxA* and *phe-2* (76 to 80% linkage) were in close agreement with those of Cuskey and Phibbs (5) for *nalA* and *phe-2* (80%

TABLE 4. Susceptibility of ciprofloxacin-resistant mutants of *P. aeruginosa* PAO2 to nonquinolone antibiotics

Strain or mutant	MIC (μg/ml)			
	Novobiocin	Carbenicillin	Chloramphenicol	Tetracycline
PAO2 (wild type)	300	50	150	50
PAO4700 (*cfxA1 cfxB1*)	1,200	200	>300	100
PAO4701 (*cfxA2*)	300	25	150	50
PAO4702 (*cfxA3*)	300	25	150	50
PAO6005 (*nalB8*)	1,200	200	>300	100
PAO4731 (*cfxA1*)	300	50	100	50
PAO4727 (*cfxB1*)	1,200	200	>300	100

TABLE 5. Concentrations of ciprofloxacin and nalidixic acid which inhibit *P. aeruginosa* DNA gyrase supercoiling activity

Source of DNA gyrase	IC_{50}[a] (μg/ml)	
	Ciprofloxacin	Nalidixic acid
PAO2 (wild type)	0.25	25
PAO4700 (*cfxA1 cfxB1*)	>8.0	1,600
PAO4701 (*cfxA2*)	>8.0	1,600
PAO236 (*nalA2*)	>8.0	1,600

[a] IC_{50} is defined as the concentration of drug which inhibits approximately half of the supercoiling activity.

TABLE 6. Concentrations of ciprofloxacin and nalidixic acid which inhibit reconstituted DNA gyrase from *P. aeruginosa* PAO2 and PAO236

DNA gyrase subunits[a]	IC_{50} (μg/ml)	
	Ciprofloxacin	Nalidixic acid
A_sB_s	0.25	25
A_sB_r	0.25	25
A_rB_s	>8.0	1,600
A_rB_r	>8.0	1,600

[a] A_sB_s, DNA gyrase from PAO2; A_rB_r, DNA gyrase from PAO236.

linkage). Because phage E79 resistance (altered lipopolysaccharide) maps in this region and exhibits cross resistance to nalidixic acid (14, 16, 21), we assayed our *cfxA* mutants for resistance to this phage. None of our mutants were resistant to E79 (data not shown). Both *cfxA* and *nalA* mutations altered subunit A of the enzyme DNA gyrase. In each case, the mutant enzyme was at least 32-fold more resistant to ciprofloxacin and 64-fold more resistant to nalidixic acid in supercoiling assays. The increase in the IC_{50}s of ciprofloxacin and nalidixic acid associated with *cfxA* mutations was similar to the increase observed in the MICs for these mutants. *cfxA* mutations resulted in an 8- to 16-fold increase in the MIC of ciprofloxacin and a 64-fold increase in the MIC of nalidixic acid. This excludes the MICs for PAO4700, which also harbors a *cfxB* mutation. Recently, Inoue et al. (12) reported that a *nalA* mutation in PAO1 increased the resistance of DNA gyrase-mediated supercoiling to quinolones. While their IC_{50}s were greater than those for ciprofloxacin and nalidixic acid obtained in this study, it is clear in each case that *nalA* and, in our case, *cfxA* mutations increase the resistance of DNA gyrase to quinolones by altering the gyrase A subunit of the enzyme. The reason for the discrepancy in IC_{50}s is unclear; however, variation in enzyme purity or assay conditions may be a factor. Scurlock and Miller (26) have also reported that *P. aeruginosa* DNA gyrase is more resistant to nalidixic acid than is *E. coli* gyrase, while Rella and Haas (23) have demonstrated that in permeabilized cells of *P. aeruginosa*, the sensitivity of DNA replication to nalidixic acid is very similar to that reported for *E. coli*. If DNA gyrase and DNA replication are equally sensitive to quinolones in *P. aeruginosa* and *E. coli*, one would suspect other reasons for the large difference in quinolone MICs for these organisms. One factor which might contribute to such a discrepancy is permeability. It is well known that *P. aeruginosa* exhibits a high intrinsic resistance to a variety of antibiotics. In the case of some hydrophilic antibiotics, such as β-lactams, this high intrinsic resistance has been predominantly attributed to the low rate of permeation of these antibiotics across the outer membrane (1, 21, 29). The mutation *cfxB1* contained in PAO4700 and PAO4727 resulted in increased resistance to quinolones as well as to several other classes of antibiotics including carbenicillin. This type of cross resistance to unrelated drugs implies an alteration in permeability. It is not surprising, therefore, that we detected alterations in the outer membrane profile of these mutants as well as the *nalB8* mutant PAO6005 (unpublished data). It is interesting that this class of mutation confers as much resistance to ciprofloxacin, norfloxacin, ofloxacin, and enoxacin as do some *cfxA* mutations, yet it confers much less resistance to nalidixic acid compared with *cfxA* mutants (Table 2). The *cfxB1* mutation increased resistance to the newer quinolones by the same proportion as it did to nalidixic acid (4- to 8-fold increase in MIC), while the *cfxA* mutants (*gyrA*) exhibited a smaller increase in resistance to the newer quinolones (8- to 16-fold increase in MIC) relative to that to nalidixic acid (64-fold increase in MIC). This is consistent with the lower incidence of resistance observed with the newer quinolones than with nalidixic acid (25). It is now clear that resistance to ciprofloxacin in *P. aeruginosa* PAO can occur by a mutation in the gene for DNA gyrase A (*gyrA*), as well as by a mutation in the *nalB* gene region. We have recently isolated secondary mutations in PAO4701 *cfxA2* by selecting for growth on 4.0 μg of ciprofloxacin per ml (unpublished data). These mutations have not been mapped but have properties similar to those of the *nfxB* mutation reported previously (10), including the appearance of a new outer membrane protein. The *cfxA* and *cfxB* mutations are distinct from the *nfxB* mutation, which maps at a different genetic locus and has a different resistance profile.

ACKNOWLEDGMENTS

We are grateful to Holly Godin, Denise Rasimas, and Nancy McPartland for performing the MIC determinations presented in this paper and to David Hooper, Paul Phibbs, Jr., and Joseph Wolf for helpful discussions and provision of bacterial strains. We also thank Roger Celesk for sharing unpublished observations on the outer membrane proteins of our mutants.

LITERATURE CITED

1. **Angus, B. L., A. M. Carey, D. A. Caron, A. M. B. Kropinski, and R. E. W. Hancock.** 1982. Outer membrane permeability in *Pseudomonas aeruginosa*: comparison of a wild type with an antibiotic super-susceptible mutant. Antimicrob. Agents Chemother. **21**:229–309.
2. **Barry, A. L., R. N. Jones, C. Thornsberry, L. W. Ayers, E. H. Gerlach, and H. M. Sommers.** 1984. Antibacterial activities of ciprofloxacin, oxolinic acid, cinoxacin, and nalidixic acid. Antimicrob. Agents Chemother. **25**:633–637.
3. **Calhoun, D. H., and T. W. Feary.** 1968. Transductional analysis of *Pseudomonas aeruginosa* methioneless auxotrophs. J. Bacteriol. **97**:210–216.
4. **Cozzarelli, N. R.** 1980. DNA gyrase and the supercoiling of DNA. Science **207**:953–960.
5. **Cuskey, S. M., and P. V. Phibbs, Jr.** 1985. Chromosomal mapping of mutations affecting glycerol and glucose catabolism in *Pseudomonas aeruginosa* PAO. J. Bacteriol. **162**:872–880.
6. **Eron, L. J.** 1987. Therapy of skin and skin structure infections with ciprofloxacin. Am. J. Med. **82**(Suppl. 4A):224–226.
7. **Fass, R. J.** 1987. Efficacy and safety of oral ciprofloxacin in the treatment of serious respiratory infections. Am. J. Med. **82** (Suppl. 4A):202–207.
8. **Feary, T. W., B. Williams, D. H. Calhoun, and T. A. Walker.** 1969. An analysis of arginine requiring mutants in *Pseudomonas aeruginosa*. Genetics **62**:673–686.
9. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50**:879–910.
10. **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.

10a. **Holloway, B. W., K. O'Hoy, and H. Matsumoto.** 1987. Recalibrated genetic map of *Pseudomonas aeruginosa*. Genet. Maps **4**:213–221.

11. **Hooper, D. C., J. S. Wolfson, E. Y. Ng, and M. N. Swartz.** 1987. Mechanisms of action of and resistance to ciprofloxacin. Am. J. Med. **82**(Suppl. 4A):12–20.
12. **Inoue, V., K. Sato, T. Fujii, K. Hirai, M. Inoue, S. Iyobe, and S. Mitsuhashi.** 1987. Some properties of subunits of DNA gyrase from *Pseudomonas aeruginosa* PAO1 and its nalidixic acid-resistant mutant. J. Bacteriol. **169**:2322–2325.
13. **Krishnapillai, V.** 1971. A novel transducing phage. Its role in recognition of a possible new host-controlled modification sys-

tem in *Pseudomonas aeruginosa*. Mol. Gen. Genet. **114**:134–143.

14. **Kropinsky, A. M. B., L. Chan, and F. H. Milazzo.** 1978. Susceptibility of lipopolysaccharide-defective mutants of *Pseudomonas aeruginosa* strain PAO to dyes, detergents, and antibiotics. Antimicrob. Agents Chemother. **13**:494–499.
15. **Lesse, A. J., C. R. Freer, R. A. Salata, J. B. Francis, and W. M. Scheld.** 1987. Oral ciprofloxacin therapy for gram-negative bacillary osteomyelitis. Am. J. Med. **82**(Suppl. 4A):247–253.
16. **Matsumoto, H., and T. Tazaki.** 1975. Serotypic recombination in *Pseudomonas aeruginosa*, p. 281–290. *In* S. Mitsuhashi and H. Hashimoto (ed.), Microbial drug resistance. University of Tokyo Press, Tokyo.
17. **Mizuuchi, K., M. H. O'Dea, and M. Gellert.** 1978. DNA gyrase: subunit structure and ATPase of the purified enzyme. Proc. Natl. Acad. Sci. USA **75**:5960–5963.
18. **Mizuuchi, K., M. Mizuuchi, M. H. O'Dea, and M. Gellert.** 1984. Cloning and simplified purification of *Escherichia coli* DNA gyrase A and B proteins. J. Biol. Chem. **259**:9199–9201.
19. **Morrison, T. G., and M. H. Malamy.** 1970. Comparison of F factors and R factors: existence of independent regulation groups in F factors. J. Bacteriol. **103**:81–88.
20. **National Committee for Clinical Laboratory Standards.** 1985. Approved standard M7-A, methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
21. **Nicas, T. I., and R. E. W. Hancock.** 1983. Outer membrane permeability in *Pseudomonas aeruginosa*: isolation of a porin protein F-deficient mutant. J. Bacteriol. **153**:281–285.
22. **Phibbs, P. V., Jr., T. W. Feary, and W. T. Blevins.** 1974. Pyruvate carboxylase deficiency in pleiotropic carbohydrate-negative mutant strains of *Pseudomonas aeruginosa*. J. Bacteriol. **118**:999–1009.
23. **Rella, M., and D. Haas.** 1982. Resistance of *Pseudomonas aeruginosa* PAO to nalidixic acid and low levels of β-lactam antibiotics: mapping of chromosomal genes. Antimicrob. Agents Chemother. **22**:242–249.
24. **Roehl, R. A., and P. V. Phibbs, Jr.** 1982. Characterization and genetic mapping of fructose phosphotransferase mutations in *Pseudomonas aeruginosa*. J. Bacteriol. **149**:897–905.
25. **Sanders, C. C., W. E. Sanders, Jr., R. V. Goering, and V. Werner.** 1984. Selection of multiple antibiotic resistance by quinolones, β-lactams, and aminoglycosides with special reference to cross-resistance between unrelated drug classes. Antimicrob. Agents Chemother. **26**:797–801.
26. **Scurlock, T. R., and R. V. Miller.** 1979. PaeExo IX: a unique deoxyribonuclease from *Pseudomonas aeruginosa* active in the presence of EDTA. Nucleic Acids Res. **7**:167–177.
27. **Staudenbauer, W. L., and E. Orr.** 1981. DNA gyrase: affinity chromatography on novobiocin-sepharose and catalytic properties. Nucleic Acids Res. **9**:3589–3603.
28. **Sugino, A., and N. R. Cozzarelli.** 1980. The intrinsic ATPase of DNA gyrase. J. Biochem. Chem. **255**:6299–6306.
29. **Yoshimura, F., and H. Nikaido.** 1982. Permeability of *Pseudomonas aeruginosa* outer membrane to hydrophilic solutes. J. Bacteriol. **152**:636–642.