# Mechanisms of Resistance to Quinolones

**George A. Jacoby**

Lahey Clinic, Burlington, Massachusetts

**The increased use of fluoroquinolones has led to increasing resistance to these antimicrobials, with rates of resistance that vary by both organism and geographic region. Resistance to fluoroquinolones typically arises as a result of alterations in the target enzymes (DNA gyrase and topoisomerase IV) and of changes in drug entry and efflux. Mutations are selected first in the more susceptible target: DNA gyrase, in gram-negative bacteria, or topoisomerase IV, in gram-positive bacteria. Additional mutations in the next most susceptible target, as well as in genes controlling drug accumulation, augment resistance further, so that the most-resistant isolates have mutations in several genes. Resistance to quinolones can also be mediated by plasmids that produce the Qnr protein, which protects the quinolone targets from inhibition. Qnr plasmids have been found in the United States, Europe, and East Asia. Although Qnr by itself produces only low-level resistance, its presence facilitates the selection of higher-level resistance mutations, thus contributing to the alarming increase in resistance to quinolones.**

Resistance to quinolones has been a problem ever since nalidixic acid was introduced into clinical medicine >40 years ago. For a time, the greater potency of the fluoroquinolones, compared with that of older quinolones, permitted complacency regarding their use, but successful treatment outcomes led to increased use, which, in turn, led to an escalating rate of resistance. In the 1990s, the use of fluoroquinolones in the United States increased by ~40%, with a doubling in the rate of resistance to ciprofloxacin among gram-negative bacilli isolated from the intensive care units of hospitals [1]. Figure 1 shows estimates of resistance in strains isolated from hospital surveys in the United States during 1997–2001. In the United States, >10% of enteric bacteria (e.g., *Enterobacter cloacae, Morganella morganii, Proteus mirabilis,* and *Serratia marcescens*) were resistant to ciprofloxacin. The rate of resistance to ciprofloxacin was even higher among *Pseudomonas aeruginosa* and related, nonenteric gram-negative pathogens. There were striking associations between resistance to quinolones and oxacillin among *Staphylococcus aureus,* resistance to vancomycin among *Enterococcus* species, and pro-

duction of extended-spectrum $\beta$-lactamase (ESBL) among *Klebsiella pneumoniae.* Even higher rates of resistance to quinolones have been reported from other parts of the world [2]. For example, during 1997–1999, ~60% of *Escherichia coli* strains isolated from hospital-acquired infections in Beijing were resistant to ciprofloxacin [6]. Although respiratory pathogens (e.g., *Moraxella catarrhalis, Haemophilus influenzae,* and *Streptococcus pneumoniae*) are generally susceptible to quinolones, resistance does occur, has been reported in localized outbreaks [7, 8], and is associated with clinical failure [9]. Resistance has also become a problem when fluoroquinolones are used to treat gonorrhea [10] and, in some parts of the world, can be a problem for the treatment of enteric infections due to *Salmonella, Shigella,* or *Campylobacter* species [11].

## TARGET-ENZYME RESISTANCE MECHANISMS

Three mechanisms of resistance to quinolones are currently recognized: mutations that alter the drug targets, mutations that reduce drug accumulation, and plasmids that protect cells from the lethal effects of quinolones [12]. The targets of quinolone action are the essential bacterial enzymes DNA gyrase and DNA topoisomerase IV [13]. Both are large, complex enzymes composed of 2 pairs of subunits. The subunits of DNA
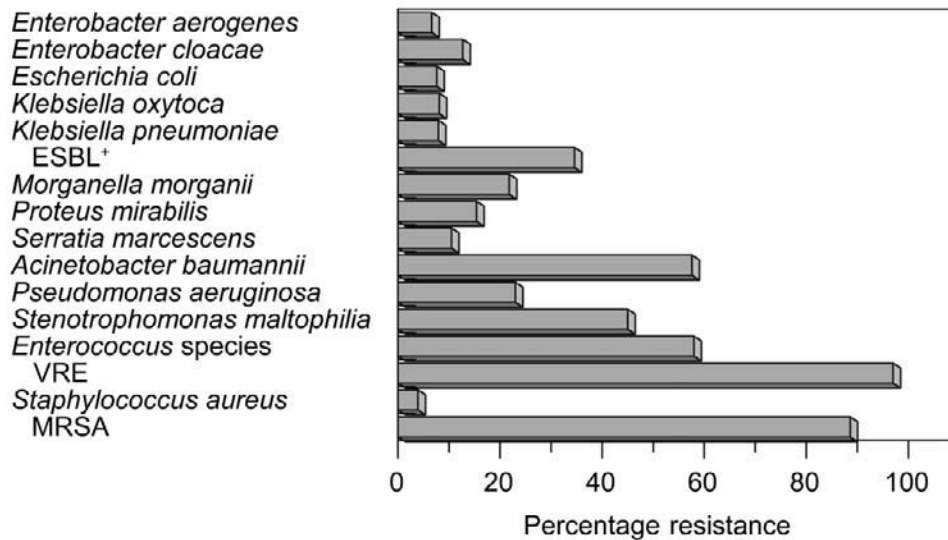
**Figure 1.** Current estimates of resistance to ciprofloxacin among isolates recovered from hospitals in the United States [2–5]. ESBL⁺, extended-spectrum β-lactamase producing; MRSA, methicillin (or oxacillin)–resistant *Staphylococcus aureus;* VRE, vancomycin-resistant enterococci.

gyrase are GyrA, a 97-kDa protein encoded by the *gyrA* gene, and GyrB, a 90-kDa protein encoded by the *gyrB* gene. The corresponding subunits of topoisomerase IV are ParC (75 kDa) and ParE (70 kDa). DNA gyrase can introduce negative supercoils into DNA, can remove both positive and negative supercoils, and can catenate and decatenate closed circular molecules. DNA topoisomerase IV can also remove positive and negative supercoils and is even better at decatenation than is gyrase. The 2 enzymes work together in the replication, transcription, recombination, and repair of DNA. The enzymes transiently break both strands of double-stranded DNA, and, in an ATP-dependent reaction, pass a second DNA double helix through the break, which is then resealed [14]. Quinolones block the reaction and trap gyrase or topoisomerase IV as a drug-enzyme-DNA complex, with subsequent release of lethal, double-stranded DNA breaks [15].

A few bacteria are able to function with only DNA gyrase, but most bacteria have both enzymes. In gram-negative bacteria, gyrase is more susceptible to inhibition by quinolones than is topoisomerase IV, whereas, in gram-positive bacteria, topoisomerase IV is usually the prime target, and gyrase is intrinsically less susceptible. Consequently, resistance mutations occur first in *gyrA* in gram-negative bacteria, but they occur first in *parC* in gram-positive bacteria. Resistance involves amino acid substitutions in a region of the GyrA or ParC subunit termed the "quinolone-resistance–determining region" (QRDR). This region occurs on the DNA-binding surface of the enzyme [16], and, for *E. coli*, DNA gyrase includes amino acids between positions 51 and 106 [17], with "hot spots" for mutation at amino acid positions 83 and 87. The QRDR in DNA gyrase is near tyrosine 122, which is covalently bound to

phosphate groups on DNA in the initial strand-breaking reaction. Conversely, in *S. aureus* or *S. pneumoniae,* the initial target mutations occur more frequently in *parC,* whereas, in highly resistant strains, additional mutations are found in *gyrA* and *parE* [18, 19].

Once a first-step mutation has reduced the susceptibility of DNA gyrase in a gram-negative organism, additional mutations in *gyrA* or mutations in *gyrB* or *parC* can further augment resistance, although, by themselves, they would be ineffective in a bacterial cell with wild-type GyrA, because the most-susceptible target sets the level of susceptibility. A plausible mechanism for how these substitutions decrease susceptibility is that they reduce drug affinity. In support of this model, single and double substitutions in the QRDR of *E. coli* gyrase have been shown to reduce the binding of quinolones to the enzyme-DNA complex [20, 21]. Alternatively, mutations may marginally impair target enzyme function and, thus, reduce the formation of enzyme-DNA complexes and lethal double-stranded breaks in DNA [22].

## EFFLUX RESISTANCE MECHANISMS

To reach their targets, quinolones must cross the cell wall and cytoplasmic membrane of gram-positive bacteria; in gram-negative bacteria, quinolones must traverse an additional outer membrane barrier. Gram-negative bacteria can regulate membrane permeability by altering expression of outer membrane porin proteins that form channels for passive diffusion, such as outer membrane proteins OmpF and OmpC in *E. coli.* In addition, both gram-negative and gram-positive bacteria have nonspecific, energy-dependent efflux systems,

some of which are expressed constitutively and others of which are controlled by global regulatory systems or are inducible by mutation. In *E. coli,* the AcrAB-TolC efflux pump plays a major role in quinolone efflux and has multiple controls. Mutations in *acrR* (a repressor of *acrAB*) increase pump activity [6]. Conversely, mutations that inactivate *marR* (a repressor of *marA*) allow MarA to activate *acrAB, tolC,* and a gene that decreases translation of *ompF,* thus collectively decreasing influx and increasing efflux of quinolones [23, 24]. Other enteric bacteria seem to be similarly equipped [25]. *P. aeruginosa* has at least 4 efflux pumps that can export quinolones and other antimicrobial agents. Efflux pumps have also been found in *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* [26, 27]. In *S. aureus,* resistance to quinolones is associated with increased expression of *norA,* a gene that encodes a broad-spectrum transporter for fluoroquinolones and other agents. Efflux contributes to resistance in *S. pneumoniae,* other gram-positive bacteria, and mycobacteria. The nonspecificity of efflux pumps also means that they can be activated in response to a variety of other compounds, including nonquinolone antibiotics, antiseptics, detergents, and even sodium salicylate [28].

Target alterations and efflux activation are often found together in resistant clinical isolates. Indeed, in *E. coli,* in the absence of the AcrAB efflux pump, *gyrA* mutations hardly increase the MICs of quinolones at all [29]. Even with a functional efflux system, single mutations in *gyrA* produce only a modest increment in resistance, such that they would be considered to be clinically susceptible (i.e., the MIC of ciprofloxacin is ⩽1 μg/mL). Only with a second mutation in *gyrA* or a mutation in *parC* is a clinical level of resistance (i.e., an MIC of ⩾4 μg/mL) reached [30, 31]. In general, the more resistant a clinical isolate, the more quinolone resistance–associated mutations it contains [32].

In principle, a quinolone that targeted both gyrase and topoisomerase IV equally would be least likely to elicit mutational resistance, because a mutation in one target would still leave the cell as susceptible as before. Target susceptibility can be measured enzymatically or genetically. Unfortunately, different approaches give different answers. For example, as measured by supercoiling or decatenation, gemifloxacin has almost equivalent inhibitory activity against purified *S. pneumoniae* DNA gyrase or topoisomerase IV, but, as measured by stabilization of the cleavable complex, topoisomerase IV is 10 times more susceptible [33]. Some investigators find that a susceptible *S. pneumoniae* isolate exposed to gemifloxacin is most likely to mutate in *gyrA,* suggesting that gyrase is the more susceptible target [34]. In *S. aureus,* gemifloxacin also targets purified gyrase and topoisomerase IV similarly in vitro, but first-step mutations arise only in topoisomerase IV, which suggests that it is the preferred target [35]. Dual-action quinolones may exist,

but, at least for gram-positive bacteria, it has been difficult to find one that meets other criteria for clinical usefulness [36].

## RATE OF EMERGENCE OF RESISTANCE TO QUINOLONES AND THE MUTANT PROTECTIVE CONCENTRATION (MPC)

Bacteria have some flexibility in setting the rate of mutation. If the rate of mutation is set too high, deleterious mutations could accumulate, but a somewhat higher-than-average rate could be an advantage for developing resistance, especially where multiple events are required [37]. When the rate of mutation of *E. coli* strains that cause urinary tract infections was compared with their MICs of quinolone or the number of quinolone resistance–associated mutations, a strong positive correlation was found in support of an association between an increased mutation rate and successful accumulation of quinolone resistance–associated mutations [32]. Certain plasmids in particular have been reported to have a mutator effect on the frequency of mutations in *E. coli* to quinolone resistance [38]. One such plasmid was recently shown to encode a DNA polymerase specialized for lesion bypass through sites of damaged DNA [39]. Quinolones themselves induce the SOS system, which leads to an elevated mutation rate [40], and quinolone resistance–associated mutations have been shown to be adaptive in the sense that they can occur in nondividing or slowly dividing cells [41].

Zhao and Drlica [42] and Drlica [43] have introduced a useful measure of the ability of bacteria to acquire resistance to quinolones by mutation. If a large inoculum ($10^{10}$ bacteria) is plated on concentrations of a quinolone that are above its MIC, and the surviving bacteria are counted after 3 days of incubation, a curve such as that shown in figure 2 is obtained. The lowest concentration at which no mutants are obtained is termed the "MPC," and the range of concentrations in which mutant selection occurs (i.e., the mutant selection window) lies between the MIC and the MPC. A double mutant could grow at a concentration greater than the MPC but would not be expected to occur in a population of $10^{10}$ bacteria. Provided that the serum or tissue concentration of a quinolone can be maintained at a level greater than the MPC, no selection of resistant mutants should occur. By this reasoning, the optimal quinolone is one with the narrowest mutant selection window, and the best schedule for drug administration is one that maintains a quinolone concentration that exceeds the MPC for as much of the dosage interval as possible, thereby minimizing the opportunity for mutant selection [42].

If an organism already has reduced susceptibility to quinolones, even if the mutation does not confer clinical resistance, then the MPC will be increased, making acquisition of higher-level resistance easier. Figure 3 shows that an *E. coli* strain with a serine-to-leucine substitution at amino acid position 83 in
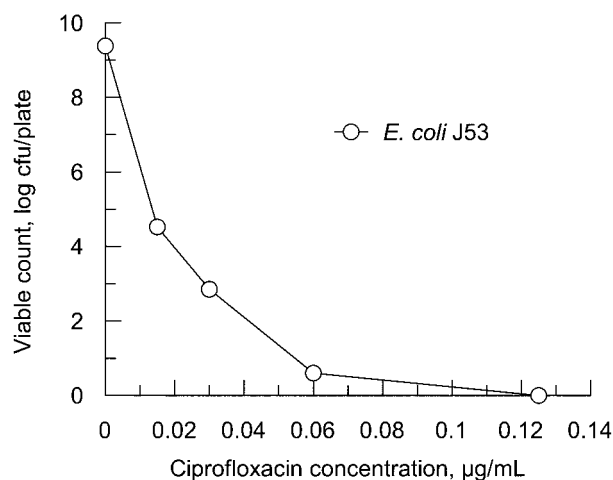
**Figure 2.** Effect of fluoroquinolone concentration on the recovery of *Escherichia coli* J53 colonies. A large inoculum ($10^{10}$ bacteria) and appropriate dilutions were applied to Mueller-Hinton agar plates containing the indicated concentration of ciprofloxacin, and surviving colonies were counted after incubation for 72 h at 37°C.

GyrA, which increases the MIC of ciprofloxacin to only 0.25 μg/mL, produced a 10-fold increase in the MPC to >1.2 μg/mL. Because the maximum serum concentration of ciprofloxacin (500 mg b.i.d.) is 2.9 μg/mL [44] and because 35% of the drug is protein bound, a strain with such a *gyrA* mutation will have a greater opportunity to acquire additional resistance mutations during therapy. Hence, prior quinolone use is a risk factor for the development of clinically significant resistance, and repeated use of the same agent increases the likelihood of therapeutic failure.

## PLASMID-MEDIATED RESISTANCE

Plasmids can also directly produce resistance to quinolones. Plasmid-mediated resistance to quinolones, long thought not to exist, was discovered in a clinical isolate of *K. pneumoniae* from Alabama that could transfer low-level resistance to quinolones to *E. coli* and to other gram-negative bacteria [45]. The plasmid increased the MIC for all quinolones tested but not for coumermycin A₁, a nonquinolone inhibitor of GyrB function. Because the plasmid did not affect intracellular concentrations of quinolones or expression of outer membrane porin and because it continued to augment resistance in *E. coli* strains with *gyrA, gyrB, parC, ompF, ompC,* or *marR* mutations, a novel mechanism of resistance was suggested [46].

The plasmid-mediated quinolone resistance gene was named "*qnr.*" The gene was cloned and was found to produce a 219-aa protein belonging to the pentapeptide repeat family [47], members of which are involved in protein-protein interactions. Purified Qnr protein was shown to bind to and protect both DNA gyrase and topoisomerase IV from inhibition by ciprofloxacin [47–49]. When a search was made for other plasmids carrying the *qnr* gene, it was not found in >300 gram-negative strains collected mainly during the 1990s. The only exceptions involved strains collected during a 6-month period in 1994 at the University of Alabama at Birmingham, where the *qnr* gene was first detected [50]. The *qnr* gene was, however, found in clinical isolates of *E. coli* from Shanghai, China, a region where isolates have high levels of resistance to ciprofloxacin [51]. Although the plasmids from Alabama and Shanghai that carried *qnr* were quite different, the gene itself was virtually identical, with only a single nucleotide change that did not alter the amino acid sequence. In further surveys of contemporary clinical strains from the United States, *qnr* has been detected in additional isolates of *K. pneumoniae* [52, 53] and, also, in *E. cloacae* [54]. It has been reported in strains from Egypt [55], Korea [56], and The Netherlands [57]; thus, *qnr* is already broadly distributed geographically. Usually, plasmids carrying *qnr* have also encoded an ESBL, such as the AmpC-type enzyme FOX-5 or the ESBLs CTX-M-9 or SHV-7. Such linkage could be one of the reasons for the high frequency of resistance to quinolones observed in ESBL-producing bacteria [58, 59].

On the plasmids that have been studied, *qnr* has been mapped in an integron or integron-like structure near an element called "Orf513." Orf513 is believed to be a recombinase involved in site-specific acquisition of resistance genes [47, 51, 60]. This location suggests that *qnr* has been acquired from some other source, but it is not known where *qnr* originated. Despite the availability of DNA sequences for an increasing number of bacterial genomes, no match with *qnr* has been found. Two members of the pentapeptide repeat family of proteins are related—at least in terms of function—to Qnr. McbG is one of the proteins produced by strains that make microcin
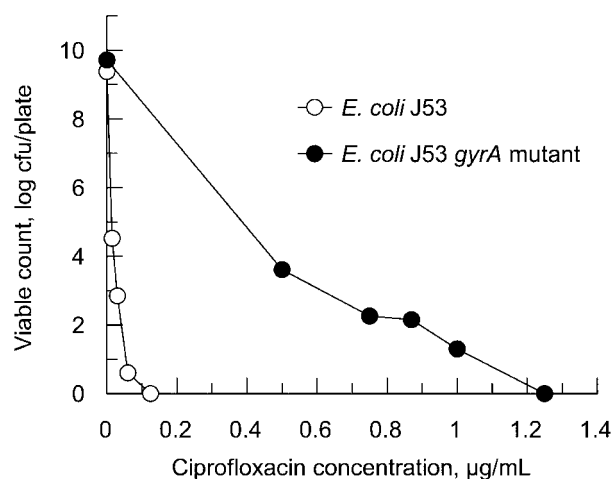


**Figure 3.** Effect of a *gyrA* mutation in *Escherichia coli* J53 on the recovery of ciprofloxacin-resistant mutants, selected as described in the legend to figure 2.
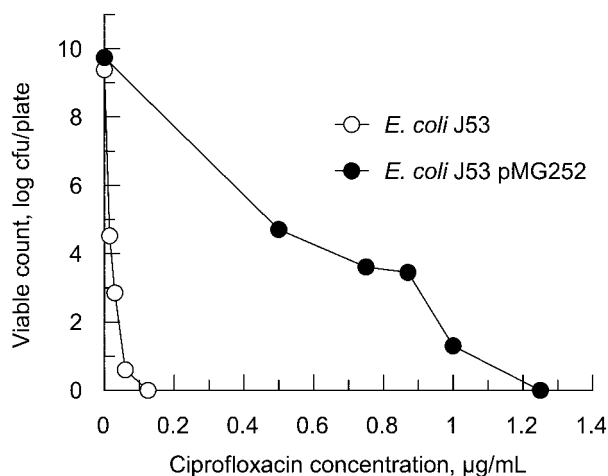
**Figure 4.** Effect of plasmid pMG252 carrying *qnr* on the recovery of ciprofloxacin-resistant mutants, selected as described in the legend to figure 2.

B17, which targets DNA gyrase. McbG is believed to protect gyrase from self-inhibition [61]. It also reduces susceptibility to some quinolones [62]. The second Qnr relative is MfpA, a protein cloned from the genome of *Mycobacterium smegmatis,* that has a 4-fold effect on susceptibility to ciprofloxacin [63]. Qnr, McbG, and MfpA have <20% amino acid identity and, thus, are not closely related, but their existence suggests that Qnr could have evolved from an immunity protein designed to protect DNA gyrase and DNA topoisomerase IV from some naturally occurring inhibitor [47].

Because Qnr by itself provides only a level of resistance to quinolones comparable to that provided by a first-step mutation in DNA gyrase (e.g., a change in the MIC of ciprofloxacin from 0.008 to 0.25 $\mu$g/mL), is such low-level resistance clinically important? Figure 4 shows how the emergence of higher-level resistance is augmented in an *E. coli* strain with a plasmid carrying *qnr*. The presence of *qnr* widens the mutant selection window, just as shown for a *gyrA* mutation in figure 3, and similarly increases the MPC of ciprofloxacin by 10-fold, thus facilitating the selection of additional resistance mutations.

The *qnr* gene seems to be a recent acquisition on plasmids, but it is not necessarily the last word in bacterial resistance to quinolones. Other members of the *qnr* family may be found. The prevalence of plasmid-encoded mutator genes could increase, although it is limited, however, by the deleterious effects of hypermutation. The ability to degrade quinolones has been described in fungi [64] and could be acquired by bacteria. Plasmid-mediated efflux systems are known for other antimicrobial agents, and genetic information for an efflux system in the same family as AcrAB-TolC has been described on a transmissible plasmid [65]. Resistance plasmids from clinical isolates can carry the microcin B17 immunity gene *mcbG,* which has slight protective activity against quinolones [50], and could

acquire genes for other proteins that were designed to protect DNA gyrase or topoisomerase IV from naturally occurring toxins but also have some antiquinolone effect. Given the persistent selective pressure for acquiring resistance to quinolones, it is likely that bacteria will continue to demonstrate their remarkable versatility in acquiring resistance to therapeutic agents.

## Acknowledgments

## References

1. Neuhauser MM, Weinstein RA, Rydman R, Danziger LH, Karam G, Quinn JP. Antibiotic resistance among gram-negative bacilli in US intensive care units: implications for fluoroquinolone use. JAMA **2003**; 289:885–8.
2. Wenzel RP, Sahm DF, Thornsberry C, Draghi DC, Jones ME, Karlowsky JA. In vitro susceptibilities of gram-negative bacteria isolated from hospitalized patients in four European countries, Canada, and the United States in 2000–2001 to expanded-spectrum cephalosporins and comparator antimicrobials: implications for therapy. Antimicrob Agents Chemother **2003**; 47:3089–98.
3. Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States, 1998 to 2001. Antimicrob Agents Chemother **2003**; 47:1681–8.
4. Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of strains expressing an extended-spectrum $\beta$-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. Clin Infect Dis **2001**; 32(Suppl 2): S94–103.
5. Hoban DJ, Doern GV, Fluit AC, Roussel-Delvallez M, Jones RN. Worldwide prevalence of antimicrobial resistance in *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis* in the SENTRY antimicrobial surveillance program, 1997–1999. Clin Infect Dis **2001**; 32(Suppl 2):S81–93.
6. Wang H, Dzink-Fox JL, Chen M, Levy SB. Genetic characterization of highly fluoroquinolone-resistant clinical *Escherichia coli* strains from China: role of *acrR* mutations. Antimicrob Agents Chemother **2001**; 45:1515–21.
7. Ho PL, Yung RW, Tsang DN, et al. Increasing resistance of *Streptococcus pneumoniae* to fluoroquinolones: results of a Hong Kong multicentre study in 2000. J Antimicrob Chemother **2001**; 48:659–65.
8. Johnson AP, Sheppard CL, Harnett SJ, et al. Emergence of a fluoroquinolone-resistant strain of *Streptococcus pneumoniae* in England. J Antimicrob Chemother **2003**; 52:953–60.
9. Davidson R, Cavalcanti R, Brunton JL, et al. Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia. N Engl J Med **2002**; 346:747–50.
10. Newman LM, Wang SA, Ohye RG, O'Connor N, Lee MV, Weinstock HS. The epidemiology of fluoroquinolone-resistant *Neisseria gonorrhoeae* in Hawaii, 2001. Clin Infect Dis **2004**; 38:649–54.
11. Low DE. Quinolone resistance and its clinical relevance. In: Hooper DC, Rubinstein E, eds. Quinolone antimicrobial agents. 3rd ed. Washington, DC: American Society for Microbiology Press, **2003**:355–86.
12. Hooper DC. Mechanisms of quinolone resistance. In: Hooper DC, Rubinstein E, eds. Quinolone antimicrobial agents. 3rd ed. Washington, DC: American Society for Microbiology Press, **2003**:41–67.

13. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev **1997**; 61:377–92.

14. Kampranis SC, Bates AD, Maxwell A. A model for the mechanism of strand passage by DNA gyrase. Proc Natl Acad Sci USA **1999**; 96: 8414–9.

15. Hiasa H, Shea ME. DNA gyrase–mediated wrapping of the DNA strand is required for the replication fork arrest by the DNA gyrase-quinolone-DNA ternary complex. J Biol Chem **2000**; 275:34780–6.

16. Cabral JHM, Jackson AP, Smith CV, Shikotra N, Maxwell A, Liddington RC. Crystal structure of the breakage-reunion domain of DNA gyrase. Nature **1997**; 388:903–6.

17. Friedman SM, Lu T, Drlica K. Mutation in the DNA gyrase A gene of *Escherichia coli* that expands the quinolone resistance–determining region. Antimicrob Agents Chemother **2001**; 45:2378–80.

18. Ng EY, Trucksis M, Hooper DC. Quinolone resistance mutations in topoisomerase IV: relationship to the *flqA* locus and genetic evidence that topoisomerase IV is the primary target and DNA gyrase is the secondary target of fluoroquinolones in *Staphylococcus aureus*. Antimicrob Agents Chemother **1996**; 40:1881–8.

19. Eliopoulos GM. Quinolone resistance mechanisms in pneumococci. Clin Infect Dis **2004**; 38(Suppl 4):S350–6.

20. Barnard FM, Maxwell A. Interaction between DNA gyrase and quinolones: effects of alanine mutations at GyrA subunit residues Ser[83] and Asp[87]. Antimicrob Agents Chemother **2001**; 45:1994–2000.

21. Willmott CJR, Maxwell A. A single point mutation in the DNA gyrase A protein greatly reduces binding of fluoroquinolones to the gyrase-DNA complex. Antimicrob Agents Chemother **1993**; 37:126–7.

22. Ince D, Hooper DC. Quinolone resistance due to reduced target enzyme expression. J Bacteriol **2003**; 185:6883–92.

23. Cohen SP, McMurry LM, Hooper DC, Wolfson JS, Levy SB. 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 **1989**; 33:1318–25.

24. Alekshun MN, Levy SB. Regulation of chromosomally mediated multiple antibiotic resistance: the *mar* regulon. Antimicrob Agents Chemother **1997**; 41:2067–75.

25. Cohen SP, Yan W, Levy SB. A multidrug resistance regulatory chromosomal locus is widespread among enteric bacteria. J Infect Dis **1993**; 168:484–8.

26. Alonso A, Martinez JL. Expression of multidrug efflux pump SmeDEF by clinical isolates of *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother **2001**; 45:1879–81.

27. Magnet S, Courvalin P, Lambert T. Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in *Acinetobacter baumannii* strain BM4454. Antimicrob Agents Chemother **2001**; 45: 3375–80.

28. Alekshun MN, Levy SB. Alteration of the repressor activity of MarR, the negative regulator of the *Escherichia coli marRAB* locus, by multiple chemicals in vitro. J Bacteriol **1999**; 181:4669–72.

29. Oethinger M, Kern WV, Jellen-Ritter AS, McMurry LM, Levy SB. Ineffectiveness of topoisomerase mutations in mediating clinically significant fluoroquinolone resistance in *Escherichia coli* in the absence of the AcrAB efflux pump. Antimicrob Agents Chemother **2000**; 44: 10–3.

30. Heisig P, Tschorny R. Characterization of fluoroquinolone-resistant mutants of *Escherichia coli* selected in vitro. Antimicrob Agents Chemother **1994**; 38:1284–91.

31. Deguchi T, Fukuoka A, Yasuda M, et al. Alteration in the GyrA subunit of DNA gyrase and the ParC subunit of topoisomerase IV in quinolone-resistant clinical isolates of *Klebsiella pneumoniae*. Antimicrob Agents Chemother **1997**; 41:699–701.

32. Komp Lindgren P, Karlsson A, Hughes D. Mutation rate and evolution of fluoroquinolone resistance in *Escherichia coli* isolates from patients with urinary tract infections. Antimicrob Agents Chemother **2003**; 47: 3222–32.

33. Yague G, Morris JE, Pan XS, Gould KA, Fisher LM. Cleavable-complex formation by wild-type and quinolone-resistant *Streptococcus pneumoniae* type II topoisomerases mediated by gemifloxacin and other fluoroquinolones. Antimicrob Agents Chemother **2002**; 46:413–9.

34. Smith HJ, Walters M, Hisanaga T, Zhanel GG, Hoban DJ. Mutant prevention concentrations for single-step fluoroquinolone-resistant mutants of wild-type, efflux-positive, or ParC or GyrA mutation-containing *Streptococcus pneumoniae* isolates. Antimicrob Agents Chemother **2004**; 48:3954–8.

35. Ince D, Zhang X, Silver LC, Hooper DC. Topoisomerase targeting with and resistance to gemifloxacin in *Staphylococcus aureus*. Antimicrob Agents Chemother **2003**; 47:274–82.

36. Smith HJ, Nichol KA, Hoban DJ, Zhanel GG. Dual activity of fluoroquinolones against *Streptococcus pneumoniae:* the facts behind the claims. J Antimicrob Chemother **2002**; 49:893–5.

37. Blázquez J. Hypermutation as a factor contributing to the acquisition of antimicrobial resistance. Clin Infect Dis **2003**; 37:1201–9.

38. Ambler JE, Pinney RJ. Positive R plasmid mutator effect on chromosomal mutation to nalidixic acid resistance in nalidixic acid–exposed cultures of *Escherichia coli*. J Antimicrob Chemother **1995**; 35:603–9.

39. Goldsmith M, Sarov-Blat L, Livneh Z. Plasmid-encoded MucB protein is a DNA polymerase (pol RI) specialized for lesion bypass in the presence of MucA′, RecA, and SSB. Proc Natl Acad Sci USA **2000**; 97: 11227–31.

40. Ysern P, Clerch B, Castano M, Gibert I, Barbe J, Llagostera M. Induction of SOS genes in *Escherichia coli* and mutagenesis in *Salmonella typhimurium* by fluoroquinolones. Mutagenesis **1990**; 5:63–6.

41. Riesenfeld C, Everett M, Piddock LJ, Hall BG. Adaptive mutations produce resistance to ciprofloxacin. Antimicrob Agents Chemother **1997**; 41:2059–60.

42. Zhao X, Drlica K. Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. Clin Infect Dis **2001**; 33(Suppl 3):S147–56.

43. Drlica K. The mutant selection window and antimicrobial resistance. J Antimicrob Chemother **2003**; 52:11–7.

44. Gonzalez MA, Uribe F, Duran Moisen S, et al. Multiple-dose pharmacokinetics and safety of ciprofloxacin in normal volunteers. Antimicrob Agents Chemother **1984**; 26:741–4.

45. Martínez-Martínez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet **1998**; 351:797–9.

46. Martínez-Martínez L, Pascual A, García I, Tran J, Jacoby GA. Interaction of plasmid and host quinolone resistance. J Antimicrob Chemother **2003**; 51:1037–9.

47. Tran JH, Jacoby GA. Mechanism of plasmid-mediated quinolone resistance. Proc Natl Acad Sci USA **2002**; 99:5638–42.

48. Tran JH, Jacoby GA, Hooper DC. Interaction of the plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. Antimicrob Agents Chemother **2005**; 49:118–25.

49. Tran J, Jacoby GA, Hooper DC. The plasmid-mediated protein Qnr protects topoisomerase IV from ciprofloxacin inhibition and interacts with the ParE subunit [abstract C1-604]. In: Program and abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago). Washington, DC: American Society for Microbiology, **2003**:71.

50. Jacoby G, Chow N, Waites K. Prevalence of plasmid-mediated quinolone resistance. Antimicrob Agents Chemother **2003**; 47:559–62.

51. Wang M, Tran JH, Jacoby GA, Zhang Y, Wang F, Hooper DC. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob Agents Chemother **2003**; 47:2242–8.

52. Rodríguez-Martínez JM, Pascual A, García I, Martínez-Martínez L. Detection of the plasmid-mediated quinolone resistance determinant *qnr* among clinical isolates of *Klebsiella pneumoniae* producing AmpC-type β-lactamase. J Antimicrob Chemother **2003**; 52:703–6.

53. Wang M, Sahm DF, Jacoby GA, Hooper DC. Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in *Klebsiella pneumoniae* clinical isolates in the United States. Antimicrob Agents Chemother **2004**; 48:1295–9.

54. Robicsek A, Sahm DF, Jacoby G, Hooper DC. Broader distribution of

plasmid-mediated quinolone resistance in the United States [abstract C2-1898b]. In: Program and abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy (Washington, DC). Washington, DC: American Society for Microbiology, **2004**:14.

55. Wiegand I, Khalaf N, Al-Agamy MHM, Wiedemann B. First detection of the transferable quinolone resistance determinant in clinical *Providencia stuartii* strains in Egypt. Clin Microbiol Infect **2004**;10s3:64.

56. Kim SH, Kwak Y, Lee M, Kim N, Jeong J, Kim Y. Plasmid-mediated quinolone resistance in clinical isolates of *E. coli* from Korea [abstract C2-1711]. In: Program and abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy (Washington, DC). Washington, DC: American Society for Microbiology, **2004**:119.

57. Paauw A, Fluit AC, Verhoef J, Leverstein-VanHall MA. A major outbreak with plasmid-mediated, qnr encoded, quinolone resistance [abstract C2-1898]. In: Program and abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy (Washington, DC). Washington, DC: American Society for Microbiology, **2004**:125.

58. Paterson DL, Mulazimoglu L, Casellas JM, et al. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum β-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. Clin Infect Dis **2000**;30:473–8.

59. Lautenbach E, Strom BL, Bilker WB, Patel JB, Edelstein PH, Fishman NO. Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*. Clin Infect Dis **2001**;33:1288–94.

60. Valentine CR, Heinrich MJ, Chissoe SL, Roe BR. DNA sequence of direct repeats of the *sull* gene of plasmid pSa. Plasmid **1994**;32:222–7.

61. Garrido MC, Herrero M, Kolter R, Moreno F. The export of the DNA replication inhibitor microcin B17 provides immunity for the host cell. EMBO J **1988**;7:1853–62.

62. Lomovskaya O, Kawai F, Matin A. Differential regulation of the *mcb* and *emr* operons of *Escherichia coli:* role of *mcb* in multidrug resistance. Antimicrob Agents Chemother **1996**;40:1050–2.

63. Montero C, Mateu G, Rodriguez R, Takiff H. Intrinsic resistance of *Mycobacterium smegmatis* to fluoroquinolones may be influenced by new pentapeptide protein MfpA. Antimicrob Agents Chemother **2001**;45:3387–92.

64. Wetzstein HG, Stadler M, Tichy HV, Dalhoff A, Karl W. Degradation of ciprofloxacin by basidiomycetes and identification of metabolites generated by the brown rot fungus *Gloeophyllum striatum.* Appl Environ Microbiol **1999**;65:1556–63.

65. Tauch A, Schlüter A, Bischoff N, Goesmann A, Meyer F, Pühler A. The 79,370-bp conjugative plasmid pB4 consists of an IncP-1β backbone loaded with a chromate resistance transposon, the *strA-strB* streptomycin resistance gene pair, the oxacillinase gene *bla*$_{NPS-1}$, and a tripartite antibiotic efflux system of the resistance-nodulation-division family. Mol Genet Genomics **2003**;268:570–84.