Mechanisms of Resistance to Quinolones

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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 production of extended-spectrum β-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
Resistance to Quinolones

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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 parE 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 glyrase 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-ToIC 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 nonquinoline 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 \(\leq 1\) \(\mu \text{g/mL}\)). Only with a second mutation in *gyrA* or a mutation in *parC* is a clinical level of resistance (i.e., an MIC of \(\geq 4\) \(\mu \text{g/mL}\)) reached [30, 31]. In general, the more resistant a clinical isolate, the more quinolone–resistance–associated mutations it contains [32].

In principle, a quinoline 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\(^\circ\)C.

GyrA, which increases the MIC of ciprofloxacin to only 0.25 \(\mu\)g/mL, produced a 10-fold increase in the MPC to >1.2 \(\mu\)g/mL. Because the maximum serum concentration of ciprofloxacin (500 mg b.i.d.) is 2.9 \(\mu\)g/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 \(A_2\), 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...
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 µg/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 anti-quinolone 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.

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