



## Fluoroquinolone Resistance : Epidemiology and Mechanism

**David Hooper**

Division of Infectious Diseases  
Massachusetts General Hospital, USA

Resistance to fluoroquinolones in gram-negative bacteria has increased over time and has been epidemiologically linked to multidrug resistance. In the United States ciprofloxacin resistance in gram-negative isolates from patients in intensive care units increased by 10% between 1990-93 to 1994-2000, with the largest increases seen for isolates of *Pseudomonas aeruginosa* (13%), *Enterobacter* (6%), and *Klebsiella* (7%). Resistance to gentamicin and ceftazidime occurred more often in ciprofloxacin-resistant than in ciprofloxacin-susceptible isolates, and in *P. aeruginosa* imipenem resistance was similarly overrepresented in ciprofloxacin-resistant strains. Similar trends in resistance and patterns of co-resistance have been seen in Europe and Asia, establishing a widespread if not worldwide trend. In addition, strains of *Campylobacter jejuni* now have a high prevalence of resistance in Europe and Central America and have been increasing in the United States. Unexpectedly, high-level resistance has also emerged in some areas of the world, including Europe and the Far East, in organisms, such as *Escherichia coli* and *Neisseria gonorrhoeae* for which resistance would have been predicted to be unlikely because of the several mutations required to meet clinical criteria for ciprofloxacin resistance.

In the case of *P. aeruginosa*, studies have linked the risks of quinolone resistance with prior quinolone exposure, and the diversity of strain types has argued for selection of new mutants in individuals receiving quinolones. In addition, however, in some settings clonal outbreaks within care units or institutions have been identified, highlighting the known capabilities of *P. aeruginosa* as a nosocomial pathogen. Similarly, risk factors for quinolone resistance in strains of *K. pneumoniae* include prior quinolone use as well as nosocomial spread. The presence of extended spectrum  $\beta$ -lactamases (ESBLs) in *K. pneumoniae* has also been shown in multivariate analyses to be a risk factor for quinolone resistance, independently of use of quinolones and third-generation cephalosporins, establishing epidemiologically a linkage of resistance to these two drug classes that extends beyond antibiotic selection pressures alone. Resistant *C. jejuni* isolates have been linked to use of fluoroquinolones in poultry, and there have been suspicions that the high rates of gastrointestinal colonization with resistant *E. coli* in parts of Europe may also be foodborne in part from contamination of poultry with ciprofloxacin-resistant *E. coli*. In Spanish outpatients, quinolone-resistant *E. coli* have been associated with use of a quinolone, urinary tract abnormalities, and use of a urinary catheter, possibly aided by a fecal reservoir of resistant isolates.

Quinolone resistance mechanisms have largely involved chromosomal mutations in the genes encoding the subunits of DNA gyrase and topoisomerase IV or in genes controlling the expression of

outer membrane porin proteins and endogenous multidrug efflux pumps. Such mutants exist spontaneously in large populations of bacteria and are selected by quinolone use. Among species, however, the level of resistance conferred by a single mutation can vary in the extent of its clinical importance. For example, a single mutation in *gyrA* of *P. aeruginosa* can produce a strain that has a ciprofloxacin MIC above the clinical breakpoint for resistance; in contrast, a single mutation in *gyrA* of *E. coli* results in strains not classified as clinically resistant but that can be the progenitor of a strain accumulating additional mutations to reach higher levels of resistance that then exceed clinical breakpoints.

Resistance mutations in *E. coli gyrA* have been shown to encode a GyrA subunit that reconstitutes a gyrase holoenzyme with decreased affinity for quinolones. Additional mutations in *gyrA* or *gyrB* likely further decrease drug affinity, and in the most highly resistant isolates, mutations are also found in *parC* and *parE* encoding subunits of topoisomerase IV, drug susceptibility levels representing the effects of quinolones on the most sensitive wildtype or mutant target enzyme within the cell. Mutations of these types have been commonly found in resistant clinical isolates of many enteric bacteria as well as in *P. aeruginosa*.

Change in efflux pump expression alone or in combination with changes in porin proteins or changes in genes known to regulate these systems have been found in resistant clinical isolates of *E. coli*, *K. pneumoniae*, *Enterobacter*, *Acinetobacter*, *Stenotrophomonas*, and in particular *P. aeruginosa*. Best studied has been *P. aeruginosa* in which changes of expression in one or more of its four efflux systems that can affect quinolone activity have been described commonly. Efflux pumps that are active on quinolones, generally also act on other antibiotics, generating resistance to multiple drugs with a profile that depends on the particular efflux pump. Interestingly, *P. aeruginosa* pumps differ in their preference for different quinolones as substrates. Notable as one example in *P. aeruginosa* is the property of the MexAB-OprM and other pumps, which when overexpressed can cause resistance to meropenem (but not imipenem) together with ciprofloxacin, possibly contributing to the linkage of those two resistances in clinical strains.

The most recently recognized mechanism of quinolone resistance in gram-negative bacteria is caused by the *qnr* gene found initially in clinical isolates of *K. pneumoniae*. The *qnr* gene has been located within integrons on plasmids, linking it to transferable multidrug resistance and particularly in association with the presence of extended spectrum or AmpC  $\beta$ -lactamases. Although conferring only low-level quinolone resistance itself, the presence of *qnr* increases the frequency of selection of chromosomal mutants that act additively with chromosomal mutations to generate strains that can cross clinical breakpoints for ciprofloxacin resistance. Qnr protein is a member of the pentapeptide repeat family of proteins that interacts with gyrase to protect it from quinolone action. Evidence has now emerged that there is a family of *qnr* proteins, *qnrA*, *qnrB*, *qnrS*, and possibly others, all of which can cause low-level quinolone resistance. The *qnr* genes have now been found on plasmids in clinical isolates of *E. coli*, *K. pneumoniae*, *Enterobacter*, *Citrobacter*, and *Shigella* with varying and in some cases substantial prevalence. Notably *qnrA* has been found in isolates classified as susceptible to ciprofloxacin, suggesting that its presence may promote the emergence of more highly resistant chromosomal mutants in clinical settings as well as in the laboratory.

With the multiplicity of quinolone resistance mechanisms at the disposal of gram-negative bacteria and the well documented and widespread emergence of resistance, protecting the future utility of

quinolones as drugs for treatment of gram-negative infections will require careful attention to drug use to limit selection and to minimize opportunities for established resistant pathogens to spread.