Parallel evolution and local differentiation in quinolone resistance in *Pseudomonas aeruginosa*

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The emergence and spread of antibiotic resistance in pathogens is a major impediment to the control of microbial disease. Here, we review mechanisms of quinolone resistance in *Pseudomonas aeruginosa*, an important nosocomial pathogen and a major cause of morbidity in cystic fibrosis (CF) patients. In this quantitative literature review, we find that mutations in DNA gyrase A, the primary target of quinolones in Gram-negative bacteria, are the most common resistance mutations identified in clinical samples of all origins, in keeping with previous observations. However, the identities of non-gyrase resistance mutations vary systematically between samples isolated from CF patients and those isolated from acute infections. CF-derived strains tend to harbour mutations in the efflux pump regulator *nfxB*, while non-CF strains tend to bear mutations in the efflux regulator *mexR* or in *parC*, which encodes one of two subunits of DNA topoisomerase IV. We suggest that differences in resistance mechanisms between CF and non-CF strains result either from local adaptation to different sites of infection or from differences in mutational processes between different environments. We further discuss the therapeutic implications of local differentiation in resistance mechanisms to a common antibiotic.

**Introduction**

Antibiotic resistance is a serious and growing obstacle to the treatment and management of microbial disease. Since the introduction of mass-produced antibiotics in the 1940s, single- and multi-drug resistant strains of most major pathogens have steadily increased in frequency (CDC, 2010; Government of Canada, 2007). Infection with a resistant pathogen increases the risk of negative patient outcomes, including mortality (Shorr, 2009), and often necessitates aggressive treatment strategies. Understanding and ultimately controlling resistance will require insights from a range of disciplines including epidemiology, molecular biology and evolutionary biology. Since resistance is, in effect, an example of adaptation in response to strong natural selection imposed by the use of antibiotics, evolutionary biology is key to addressing the problem of resistance. Under discussion here are some of the most central and long-standing problems in evolution: understanding the rate and extent of adaptation, the variety of genetic targets underlying adaptation, and the specificity of adaptation.

In this review, we discuss the evolution of quinolone resistance, focusing on the opportunistic pathogen *Pseudomonas aeruginosa*. We summarize published surveys on the mechanisms of resistance identified in clinical samples, and find that mutations in *gyrA*, which encodes the primary target of quinolones, are most common. This observation has been made previously by a number of other studies in *P. aeruginosa* and other bacterial species, and so constitutes an example of parallel evolution at the molecular level. Notably, though, we also find evidence that additional resistance mutations differ between *Pseudomonas* strains sampled from the lungs of cystic fibrosis (CF) patients and those sampled from acute, non-CF infections. We discuss both of these findings – highly parallel evolution and local differentiation – in the light of evolutionary theory, and suggest further studies and therapeutic implications.

**Quinolone targets and mechanisms of resistance**

Quinolone antibiotics constitute a widely used class of broad spectrum antibiotics first discovered in 1962 and introduced for clinical use in 1967 as a treatment for urinary tract infections caused by enterococcal bacteria (Emmerson & Jones, 2003). Following the introduction of the first quinolone antibiotic, nalidixic acid, subsequent work led to the development of fluoroquinolones (e.g. ciprofloxacin), a large and effective family of compounds. Quinolones target two paralogous enzyme complexes: DNA gyrase and DNA topoisomerase IV. Each complex is made up of two subunits: GyrA and GyrB in the case of gyrase, and ParC and ParE in the case of topoisomerase IV. The gene duplication events that gave rise to these two complexes are very ancient, predating the split between...
Gram-negative and Gram-positive bacteria (e.g. Sissi & Palumbo, 2010). DNA gyrase and topoisomerase IV play important roles in DNA supercoiling and in the decatenation of closed circular DNA molecules. As such, they function in a number of cellular processes, including DNA replication, transcription and recombination (reviewed by Nöllmann et al., 2007; Schoeffler & Berger, 2005).

Quinolones trap DNA gyrase or topoisomerase IV in a complex with cleaved DNA, blocking DNA replication and eventually leading to cell death. Quinolone resistance can be acquired via mutations that reduce drug binding without abolishing normal enzymic functions (Chen & Lo, 2003; Kureishi et al., 1994). A number of mutations in the quinolone-resistance-determining regions (QRDR) of gyrase and topoisomerase IV can grant resistance in this manner; the most important of these mutations occurs at position 83 of GyrA, which corresponds to residue 80 in ParC (Fig. 1a; all residue numbering follows the *P. aeruginosa* positions, which match the canonical *Escherichia coli* positions). These residues are highly conserved throughout prokaryotes (Fig. 1b), with serine and threonine as the most common amino acids. Mutations to leucine (from serine) or isoleucine (from threonine), and less frequently to alanine, have been reported in clinical and *in vitro* studies as conferring resistance (reviewed by Piddock, 1999). Additional mutations at residue 87 of GyrA (Fig. 1a; residue 84 of ParC), as well as in GyrB and ParE, also contribute to resistance, and are frequently observed in combination with S83L or T83I mutations (Chen & Lo, 2003).

In addition to mutations in target enzymes, quinolone resistance can be gained via a reduction in intracellular drug concentration, either by reducing influx or by increasing efflux. In Gram-negative bacteria, a reduction in influx can be achieved by mutations in porin structural genes. Porins form channels in the bacterial membrane, through which exogenous molecules can enter the cell. Thus, a decrease in the number of porin channels serves to reduce entry of antibiotic into the cell (Jacoby, 2005).

Increased efflux of antibiotic in *P. aeruginosa* is typically achieved by the upregulation of chromosomally encoded efflux pumps. Efflux pump repertoires vary widely among different bacterial species, such that different mutations are responsible for quinolone resistance in different taxa. In *P. aeruginosa*, at least two efflux pumps are thought to play important roles in quinolone resistance: MexAB–OprM and MexCD–OprJ (Poole, 2005). Each pump is encoded by a single operon, and this operon is repressed by a single regulatory protein. Loss-of-function mutations in the gene encoding the regulatory protein thus serve to increase efflux pump expression, leading to decreased drug susceptibility. In *P. aeruginosa*, the *mexR* and *nfxB* genes encode repressors of MexAB–OprM and MexCD–OprJ, respectively, and mutations in these genes can increase pump expression (e.g. Poole, 2005).

The resistance mechanisms described thus far are all examples of chromosomal resistance mutations. In the last 13 years, however, a number of plasmid-borne quinolone resistance genes have been identified, primarily in *E. coli* and in *Klebsiella pneumoniae* (reviewed by Strahilevitz et al., 2009). Plasmid-borne resistance genes are transferred horizontally between bacteria, and thus represent the potential for the rapid spread of resistance, both within and among species. None of these genes has been reported in a clinical isolate of *P. aeruginosa*, although Martínez-Martínez et al. (1998) demonstrated that the *qnr* gene does confer resistance in this species if introduced via conjugation. As such, for the remainder of this paper, we restrict our discussion to chromosomal resistance determinants.

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**Fig. 1.** (a) The structure of *E. coli* DNA gyrase, with S83 highlighted orange and D87 highlighted red. (b) Conservation of the DNA binding pocket in GyrA. Residues 83 and 87 are highlighted in orange and red, respectively. The phylogeny was reconstructed from the full amino acid sequence of GyrA.
A survey of clinically relevant quinolone resistance mutations

The widespread use of quinolones has led to a rapid increase in the incidence of resistance in many pathogenic bacteria. Prevalence of resistance to ciprofloxacin, a commonly used fluoroquinolone, ranges from 5% to over 80% amongst hospital isolates of important nosocomial pathogens (Jacoby, 2005). Given the prevalence of quinolone resistance, considerable effort has been devoted to identifying resistance mutations in clinical isolates of many pathogens.

In order to quantitatively ascertain the frequency and importance of different resistance mutations, we have undertaken a survey of known resistance mutations reported in quinolone- and/or fluoroquinolone-resistant isolates of *P. aeruginosa*. *P. aeruginosa* is a Gram-negative bacterium that occupies a broad range of environmental and pathogenic habitats. Importantly, it has the capacity to cause radically different acute and chronic infections. *P. aeruginosa* infects a number of sites, including the urinary tract, eye, burn wounds, lung and blood. It is responsible for about 10% of acute hospital-acquired infections, where it can cause acute pneumonia in hospitalized patients (Hancock, 1998). In addition, *P. aeruginosa* is a major pathogen of individuals with CF. In Canada, for example, it occurs in 60–70% of adults with CF (Stephenson, 2008). Both population-based and case-control studies have demonstrated that infection with *P. aeruginosa* appears to be an independent prognostic factor that carries with it an increased risk of death in CF patients, irrespective of lung function (Corey & Farewell, 1996; Rosenfeld *et al.*, 1997).

We identified 16 publications that investigated the molecular mechanism(s) of resistance in one or more clinical isolates of *P. aeruginosa*. Of these 16 studies, 13 examined only isolates from non-CF patients, two examined only CF isolates and one examined isolates from both CF and non-CF patients. Most studies identified resistance mutations by direct sequencing of candidate loci, or by genotyping specific SNPs; a few studies examined efflux pump mRNA levels or used complementation tests to identify gyrA mutants. The full datasets are given in Supplementary Table S1, available with the online version of this paper. Interestingly, we find evidence of both widespread parallel evolution and local differentiation amongst documented mechanisms of antibiotic resistance. These patterns give hints regarding the processes underlying the evolution of antibiotic resistance, and suggest further avenues of research.

Parallel evolution in quinolone resistance

Parallel evolution is the evolution of the same genotypes and/or phenotypes repeatedly and independently. The observation of parallel evolution as a response to similar ecological pressures is a powerful indication that natural selection is responsible for the evolution of a given trait or genotype. Parallel evolution has historically been discussed in the context of whole organism studies of adaptive evolution where it is used to infer patterns of past selection. For example, populations of three-spine sticklebacks (a widespread species of fish) have independently colonized freshwater lakes from the ocean hundreds or thousands of times since the last ice age. Colonization events are typically accompanied by a suite of parallel morphological changes, including reductions in body armour and spine length. The consistency with which these changes occur upon freshwater colonization is strong evidence that they are driven by natural selection, rather than another process, e.g. genetic drift (e.g. Bell & Foster, 1994). In microbes, parallel evolution is less well studied, although it is known to occur in the context of metronidazole resistance in *Helicobacter pylori* (Albert *et al.*, 2005). However, the simple fact that we ‘know’ the genetic targets of resistance for the most commonly used antibiotics, and that these are highly conserved across species (for example, gyrA mutations confer resistance to quinolones, rpoB mutations confer resistance to rifampicin and so on), suggests that parallel evolution is a hallmark of resistance evolution.

We find a marked pattern of parallel evolution with respect to quinolone resistance in *P. aeruginosa*. We find that mutations in gyrA are very common amongst resistant strains, regardless of the type of infection (CF vs non-CF) (Fig. 2), in accordance with previous suggestions (Henrichfreise *et al.*, 2007). Similar patterns have been observed in numerous other species, with gyrA mutations common in Gram-negative bacteria (Piddock, 1999).

Recent genetic screens in *E. coli* (Tamae *et al.*, 2008) and *P. aeruginosa* (Breidenstein *et al.*, 2008) have identified genes whose knockouts either reduce or increase sensitivity to ciprofloxacin, with >100 and 40 genes, respectively, contributing to resistance. Most of these mutations have not been genotyped in clinical samples, so that their contributions to clinical resistance cannot yet be fully determined. Nonetheless, the overwhelming prevalence of gyrA mutations suggests that regardless of how many other potential mutations could contribute to resistance, the gyrA mutants are favoured. Part of the reason for the selection of gyrA mutations is probably the greater reduction in susceptibility that they afford. In *P. aeruginosa*, a 16–70-fold increase in MIC can be attributed to the T83I mutation (Cambau *et al.*, 1995; Diver *et al.*, 1991; Kureishi *et al.*, 1994). By contrast, the transposable element mutations described by Breidenstein *et al.* (2008) result in much more modest (2–4×) increases in MIC. Thus, even though a gyrA T83I variant is no more likely to arise in a population than any other resistance (point) mutation, the fitness benefit that it affords in the presence of a high concentration of antibiotic virtually guarantees its success once it does arise. At lower antibiotic concentrations, however, other mutations could play important roles (Zhou *et al.*, 2000).

Other factors may also help to explain the prevalence of gyrA mutations. The costs of resistance may be particularly
important in understanding why some resistance mutations are prevalent, and why they persist following the cessation of antibiotic treatment. Resistance mutations, while beneficial in the presence of antibiotic, may inflict fitness costs in the absence of drug (Andersson, 2006; Perron et al., 2010; Ward et al., 2009). Such costs arise through pleiotropic effects of the mutation on other traits important to fitness, such as growth rate. Once antibiotic treatment has finished, costly resistance mutations should be rapidly eliminated from a population by genotypes bearing the wild-type, non-costly allele. Such genotypes could be introduced anew into the population from environmental sources, or by back-mutation from the mutant type. However, resistance is often maintained even after treatment has run its course and antibiotic use has been terminated (Andersson, 2006). The maintenance of resistance could be explained by no-cost resistance mutations that have wild-type (or better) fitness in the absence of antibiotic. Alternatively, second-site mutations may compensate for the costs of resistance, while maintaining the resistance afforded by the original mutation. The available data are mixed with respect to the costs of resistance to fluoroquinolones, although there is a general pattern that commonly found S83L/T83I mutations do bear a cost. Bagel et al. (1999) found that E. coli strains bearing single S83L or D87G mutations in gyrA had a 33% longer doubling time than did isogenic wild-type strains, and that double mutants carrying both mutations had a 104% increase in doubling time. Similarly, Kugelberg et al. (2005) found a reduced growth rate for P. aeruginosa bearing the T83I mutation. Interestingly, however, other nalidixic-acid-resistant mutants isolated in the Kugelberg study, including D87G and T83A, suffered no substantial cost (these mutants also had higher susceptibility than T83I mutants). Moreover, the fitness cost of the T83I mutation could be compensated after only 20–30 generations of serial passaging in permissive conditions. Kassen & Bataillon (2006) identified between 10 and 28 nalidixic-acid resistant mutants (out of 665 resistant mutants screened) of Pseudomonas fluorescens that were fitter than the wild-type strain in permissive media. The uncertainty associated with the number of mutants reflects how statistically stringent one wishes to be in defining a mutant as fitter than the wild-type. Preliminary work has shown that mutations at codons 83 and 87 of gyrA predominate among these mutants, although a comprehensive screen has yet to be conducted. In general, if S83L/T83I mutations are costly, then compensatory second-site mutations may play an important role in their prevalence and persistence.

**Environment-specific mechanisms of resistance in P. aeruginosa**

In our literature survey, mechanisms of quinolone resistance differed between P. aeruginosa samples taken from CF and non-CF patients. For both groups, mutations in gyrA are the most common amongst resistant clinical strains. However, frequencies of additional resistance mutations vary significantly between the two types of sample (Fig. 2): amongst samples from CF patients, 57% of resistant strains bear mutations in nfxB, which acts as a negative regulator of the MexCD–OprJ efflux pump. None of the CF strains carried mutations in parC or in mexR. Amongst non-CF samples, however, the situation is reversed: 48 and 33% bear mutations in parC and mexR, respectively, while only 16% carry nfxB mutations. Thus, these data suggest an important role for nfxB-mediated resistance in samples from CF patients, with a major role for parC mutations, and for different efflux mechanisms in non-CF samples. These patterns have been hinted at in earlier studies (Henrichfreise et al., 2007; Jakics et al., 1992), but this is the first systematic analysis, to our knowledge, of quinolone resistance mutations between CF and non-CF isolates.

These observations raise the question as to why different resistance mutations are seen in different types of infection, that is, how evolutionary outcomes are dictated by environmental differences between the CF lung and sites of acute infection. It is becoming increasingly clear that the
unique environment of the CF lung plays an important role in determining the physiological state and evolutionary trajectory of *P. aeruginosa* during chronic infection. Lungs of CF patients become filled with viscous sputum, and the viscosity, nutritional composition and low aerobity of CF sputum are important and peculiar features of this microbial habitat (Brown *et al.*, 2008; Hassett *et al.*, 2009; Worlitzsch *et al.*, 2002). In the present context, such features of the CF lung must influence either (or both) the processes of mutation and selection in order to account for differences in drug resistance mechanisms.

Differences in mutational patterns between environments could account for the different resistance mutations if, for example, mutations at *nfxB* occur more often in the CF lung than elsewhere. While descriptions of similar environment-specific variation in mutational patterns are rare, Björkman *et al.* (2000) argued for their occurrence during compensatory evolution in *Salmonella typhimurium*. They found different mutations in streptomycin-resistant strains that had been allowed to undergo compensatory evolution in LB or in mice. The fitness effects of the different mutations did not differ between the two environments, leaving differential mutational processes as the best explanation for the observation. Similarly, it is possible that differences in the spectrum of quinolone resistance mutations among strains isolated from CF and non-CF patients are the result of systematic variation in mutational processes.

Alternatively, the fitness effects of different mutations may be environment-specific, such that the unique culture conditions presented by the CF lung favour *nfxB* mutations over *parC* or *mexR* mutations, owing to the level of resistance granted and/or any negative, indirect consequences of resistance (e.g. reduced growth rate). This alternative hypothesis thus invokes selection rather than mutation. A difference in the rank order of fitness effects of mutations by environment is often referred to as a genotype-by-environment (GxE) interaction in evolutionary genetics. GxE interactions have been the subject of much theoretical and empirical work in evolutionary biology (e.g. Dean, 1995; Hedrick, 2006; Remold & Lenski, 2001), and the existence of GxE interactions has important implications for the maintenance of polymorphism and for local adaptation. Genetic variation can be supported if different mutations are favoured in different environments, and this situation will lead to populations that are well adapted to their local environments, but not necessarily to other conditions.

Work on GxE interactions draws attention to three distinct mechanisms by which differential fitnesses could be generated for *P. aeruginosa* drug resistance mutations. Broadly, differences in the strength of selection, or in either the physiological state or the genetic background of *P. aeruginosa* in CF and non-CF infections, may underlie differences in fitness ranking. We will refer to these three mechanisms as the ‘effective dose’, ‘plasticity’ and ‘epistasis’ hypotheses.

**Hypothesis 1: effective dose**

Drug concentrations can vary dramatically amongst tissues and, as previously noted, different resistance mutations can be selected at different antibiotic concentrations (Zhou *et al.*, 2000). Studies of *in vivo* ciprofloxacin concentrations in CF patients have found systematically lower drug levels in sputum than in blood (sputum concentrations of 0.2–1.9 mg l⁻¹ versus blood concentrations of 2.4–4 mg l⁻¹, depending on dose and study; Pedersen *et al.*, 1987, and references therein). Thus, variations in drug resistance mechanisms may reflect these local differences in concentration, if, for example, *nfxB* mutations confer a modest decrease in susceptibility with few negative consequences. Ciprofloxacin concentrations in other tissues in non-CF patients also vary widely, however (e.g. 1.1 mg l⁻¹ in adipose tissue, 2 mg l⁻¹ in lung epithelial lining fluid, 3 mg l⁻¹ in the prostate; Hoogkamp-Korstanje *et al.*, 1984; Joukhadar *et al.*, 2005; Winter & Sweeney, 1991). Thus, it is not clear whether the prevalence of *nfxB* mutations in CF samples are attributable to differences in drug concentration alone.

**Hypothesis 2: plasticity**

*P. aeruginosa* undergoes a number of physiological changes in the CF lung, such as growth as an unattached biofilm or microcolony, in contrast with its planktonic lifestyle in other types of infection (Worlitzsch *et al.*, 2002). Such physiological changes can be referred to as ‘plastic’ insofar as they do not result from genetic mutations, but rather from cellular responses to the environment. The carbohydrate matrix surrounding microcolonies may decrease drug penetration, and biofilm-specific efflux mechanisms (e.g. Zhang & Mah, 2008) involving additional efflux pumps can reduce intracellular drug concentrations. If the level of resistance afforded by *nfxB* mutations is lower than that afforded by *parC* and/or *mexR* mutations, but with fewer indirect effects for *nfxB* mutations, then *nfxB* mutants would be expected to benefit from the biofilm growth typical of CF infections. Other physiological changes associated with the CF lung may also play important roles, such as decreased metabolic activity in biofilm colonies (Høiby *et al.*, 2010).

**Hypothesis 3: epistasis**

While some of the physiological changes associated with the CF lung habitat are likely to be purely plastic, other alterations are known to have a genetic basis. Following colonization of the CF lung, *P. aeruginosa* undergoes a number of repeatable genetic changes, such as mutations to *lasR* and *mucA* (D’Argenio *et al.*, 2007; Mathee *et al.*, 2008; Rau *et al.*, 2010; Rowen & Deretic, 2000), that have not been reported in other types of infection. Epistatic interactions between quinolone-resistance mutations and these presumably CF-adaptive mutations may therefore underlie differences in patterns of clinical resistance mutations. For example, *nfxB* mutations may have an
advantage when present on a lasR mutant background, with mexR and/or parC mutants most fit on a wild-type lasR background.

Testing the hypotheses

We have suggested four hypotheses (mutation, effective dose, plasticity and epistasis) to account for different mechanisms of quinolone resistance between CF and non-CF strains of *P. aeruginosa*. Currently, data are not available to directly test these hypotheses, but several experimental approaches could be useful in distinguishing amongst them.

Selective and mutational explanations for differences in quinolone resistance mechanisms offer differing predictions with respect to the fitness effects of resistance mutations in CF and non-CF culture conditions. If differences in mutational spectra are driven by selection, then one would expect nfxB mutations to be favored under conditions resembling the CF lung, with mexR and/or parC favored under conditions resembling acute infections. In contrast, a mutational mechanism would be suggested if no differences in fitness were observed.

Further testing of the mutational hypothesis could be obtained by characterizing the mutational profile of *P. aeruginosa* under different environmental conditions. Spontaneous mutants can be isolated by using fluctuation assays (Kassen & Battaillon, 2006; Luria & Delbrück, 1943) or by mutation accumulation (Halligan & Keightley, 2009). Identification of mutations in a large number of mutants isolated under CF-like and non-CF-like conditions would serve to test the hypothesis that mutational processes differ between these two environments.

Several approaches can contribute to distinguishing between the three possible selective hypotheses. For example, selection of mutants at different quinolone concentrations – chosen to reflect known concentrations in patients – would test the hypothesis that nfxB mutants are more prevalent at lower drug concentrations. A previous study in *Mycobacterium* has shown that gyrA mutations are more likely to be selected at high quinolone concentrations, with non-gyrA mutations prevalent at low concentrations (Zhou et al., 2000). However, this study did not identify the non-gyrA mutations, and so does not bear directly on the differences in non-gyrA mutations described here.

With respect to plasticity, genetic approaches should help to identify genes that are important for quinolone resistance in CF-like conditions; such genes may encode inducible efflux pumps [such as those described by Zhang & Mah (2008) for tobramycin resistance], proteins important for biofilm formation and other physiologically regulated molecules (e.g. Fung-Tomc et al., 1993; Palmer et al., 2007). For any gene contributing to GxE interactions, the change in rank order of fitness of nfxB, mexR and parC mutations should be dependent on its expression. Thus, for example, rank order of fitness would not be expected to change in a genotype where an inducible efflux pump had been knocked out or repressed, if upregulation of this pump were responsible for the GxE interaction.

Finally, studies of adaptation to the CF lung have identified a number of loci that are often mutated in CF infections (e.g. lasR, mucA), and these loci are good candidate genes that could have epistatic relationships with nfxB, mexR or parC. Epistasis between quinolone resistance mutations and mutations beneficial in the CF lung can be evaluated by generating all possible mutant combinations (for example, all four possible genotypes bearing wild-type or mutant lasR and wild-type or mutant nfxB) on an isogenic background, and assaying fitness using standard competitive or non-competitive techniques. If epistasis causes GxE interactions, one would expect (for example) lasR, nfxB double mutants to have the highest fitness under CF-like conditions, and wild-type lasR, mexR mutant genotypes to have the highest fitness under conditions characteristic of acute infections.

Conclusions

The increasing prevalence of single- and multi-drug resistant pathogens necessitates the development of carefully planned antibiotic treatment regimens. Importantly, the occurrence of environment-specific mechanisms of resistance complicates treatment and mitigation strategies. Approaches such as switching to new antibiotics or using multi-drug cocktails might be effective under one set of conditions but not another, depending on the identity of the second site mutations. In *P. aeruginosa*, the MexAB–OprM efflux pump (regulated by mexR) extrudes a number of antibiotics, including quinolones, macrolides and β-lactams (Masuda et al., 2000). The MexCD–OprJ efflux pump (regulated by nfxB) also extrudes a variety of antibiotics, but it is ineffective against some β-lactams, such as cephems. Thus, these β-lactams may be unsuitable for use against quinolone-resistant *P. aeruginosa* in acute infections but may be suitable for CF patients. The phenomenon of local differences in resistance mechanisms (whether driven by mutation or selection) should be addressed during the design of treatment regimens: if different host environments tend to favor different resistance mutations, then the choice of drug combinations should reflect this fact.

Other bacterial species may also evolve environment-specific resistance mechanisms. *Staphylococcus aureus*, like *P. aeruginosa*, can cause acute infections as well as chronic biofilm infections in CF patients. It would be very interesting to determine whether *S. aureus* strains isolated from CF and non-CF patients show analogous similarities and differences in mechanisms of quinolone resistance. At this time, however, there have been insufficient studies on mechanisms of quinolone resistance in *S. aureus* to make this comparison.

The management of antibiotic resistance at both the patient and population level is a complex and difficult
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problem, necessarily drawing on a wide range of disciplines. Because pathogen populations are subject to intense natural selection by antibiotics, it is important to understand the evolutionary patterns and processes underlying resistance. For example, we have highlighted here the potential roles of biased mutation and GxE interactions. Ultimately, predicting the evolutionary responses of microbial populations to antibiotic treatment will be a necessary component of controlling the emergence, spread and persistence of resistant pathogens.

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References


