

## Mechanisms of quinolone action and microbial response

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**Over the years, chromosomal mapping of the bacterial genome of *Escherichia coli* has demonstrated that many loci are associated with quinolone resistance, which is mainly a result of chromosomal mutation or alteration of the quantity or type of porins in the outer membrane of Gram-negative bacteria. There has been one report of a small and confined episode of plasmid-mediated resistance to fluoroquinolones, which did not appear to persist. With the increasingly widespread use of an expanding range of fluoroquinolone antibiotics, a range and mix in individual bacterial isolates of the different mechanisms of resistance to fluoroquinolones will undoubtedly be encountered amongst clinically significant bacteria. Currently, transferable resistance is extremely rare and most resistant bacteria arise from clonal expansion of mutated strains. However, it is conceivable that in the future, horizontal gene transfer may become a more important means of conferring resistance to fluoroquinolones.**

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### Overview of the targets for the quinolones

The chromosome of *Escherichia coli* is composed of a single, double-stranded DNA circle greater than 1000 µm in length. In order for it to be accommodated within a bacterial cell that is only 1–3 µm long, the chromosomal DNA undergoes tertiary folding and compaction to reduce its volume. This is achieved by: macromolecular crowding created by the high concentration of solutes in the cytoplasm; long-range DNA folding into 50–80 topologically independent domains; negative DNA supercoiling; and the presence of small DNA bending proteins. DNA is synthesized at a rate of 50 000 bp/min/replication fork and in order that replication occurs efficiently, the DNA strands must readily separate, and tangles, precatenates (crossovers) and catenates (intermolecular links) must be rapidly removed. *E. coli* produces enzymes called DNA topoisomerases that control the topology of the chromosomal DNA to facilitate replication, recombination and expression. Two of these enzymes, DNA gyrase and the more recently discovered DNA topoisomerase IV, are targets of the fluoroquinolones.<sup>1</sup>

### DNA gyrase

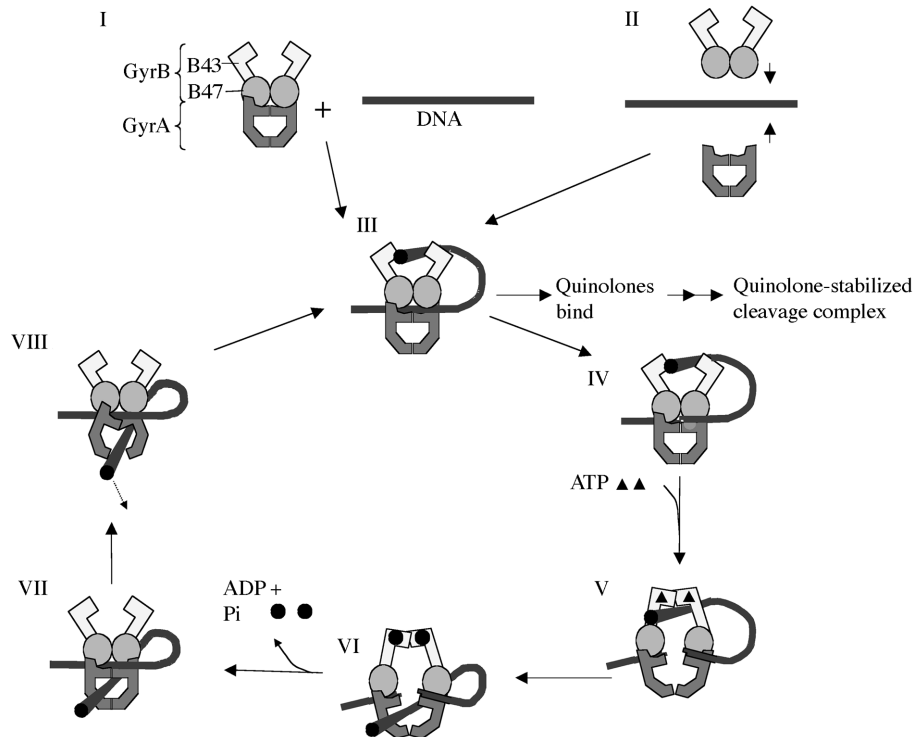
DNA gyrase is a tetramer of two A and two B subunits, encoded by the *gyrA* and *gyrB* genes, respectively. *gyrA* and

*gyrB* are located at 48 and 83 min, respectively, on the *E. coli* genome, but in some bacteria they are adjacent to each other and *oriA*; this configuration is known as the quinolone resistance determining region (QRDR).<sup>2,3</sup> DNA gyrase is responsible for introducing negative supercoils into DNA and for relieving topological stress arising from the translocation of transcription and replication complexes along DNA. It acts by wrapping DNA into a positive supercoil and then passing one region of duplex DNA through another via DNA breakage and rejoining (Figure 1). This is an ATP-dependent process. In the presence of ATP, the process is driven forward, increasing supercoiling. In the absence of ATP, the process is driven back again, relaxing the DNA. Keeping the DNA chromosome wound into loops facilitates the movement of replication forks.

### Topoisomerase IV

Topoisomerase IV is a homologue of DNA gyrase, comprising four subunits, two of C and two of E, encoded by the *parC* and *parE* genes, respectively. The topoisomerase IV locus was described in 1990.<sup>4</sup> However, a number of quinolone resistance markers had already been described and mapped to this locus. In *Staphylococcus aureus*, the *flq* locus, now referred to as *grlA*, is equivalent to *parC* in other bacteria such

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**Figure 1.** Schematic illustration of the DNA gyrase supercoiling cycle, showing the point of action of quinolones. The domains of gyrase are shown in different grey shades with the C-terminal (DNA-wrapping) domain of GyrA omitted for clarity. Figure provided by Dr J. G. Heddle and Professor A. Maxwell, John Innes Centre, Norwich, UK.

as *Streptococcus pneumoniae*. In *E. coli*, the *nfxD* locus is now *parE*. The reaction mechanism of topoisomerase IV is similar to that of gyrase but topoisomerase IV binds to DNA crossovers rather than wrapping DNA. Topoisomerase IV is primarily involved in decatenation, the unlinking of replicated daughter chromosomes.

### Interruption of gyrase activity by quinolones

Quinolones act by binding to complexes that form between DNA and gyrase or topoisomerase IV. Shortly after binding, the quinolones induce a conformational change in the enzyme. The enzyme breaks the DNA and the quinolone prevents re-ligation of the broken DNA strands. The enzyme is trapped on the DNA resulting in the formation of a quinolone–enzyme–DNA complex. Quinolone–gyrase–DNA complex formation rapidly inhibits DNA replication and is consistent with gyrase acting ahead of replication forks. However, inhibition of replication by quinolone–topoisomerase IV–DNA complexes occurs slowly, consistent with the enzyme being located behind the replication forks.<sup>5</sup> Complex formation reversibly inhibits DNA and cell growth and is thought to be responsible for the bacteriostatic action of the quinolones. Lethal action is not reversible and is thought to be a separate event from complex formation. Higher quinolone concen-

trations are required to kill cells rather than to inhibit growth or form complexes and some quinolones inhibit growth better than others but are less effective at killing.<sup>6</sup>

It is thought that cell death arises from the release of DNA ends from quinolone–gyrase–DNA complexes (Figure 1). Evidence that the release of free DNA ends, not simply the formation of complexes, accounts for the bactericidal action of quinolones, derives from sedimentation analysis of isolated bacterial nucleoids.<sup>7</sup>

However, the lethal action of oxolinic acid can be blocked by chloramphenicol, an inhibitor of protein synthesis, but only a partial effect is seen with ciprofloxacin. This implies that a protein factor is involved in the release of free DNA ends from quinolone–gyrase–DNA complexes and that some quinolones have an additional chloramphenicol-insensitive mode of action. Little is known about the protein factor and it has been suggested that the chloramphenicol-insensitive mode of action may arise from the stimulation of gyrase subunit dissociation.<sup>7</sup>

### Resistance mechanisms related to topoisomerase inhibition

Resistance to quinolones most commonly arises stepwise as a result of mutations usually accumulating in the genes encod-

ing DNA gyrase and topoisomerase IV. Analysis of MIC values for resistant clinical isolates, and direct studies of the inhibitory action of quinolones on DNA gyrase and topoisomerase IV activity have demonstrated that in Gram-negative species such as *E. coli* and *Neisseria gonorrhoeae*, the primary target of the quinolones is gyrase, whereas in Gram-positive organisms, such as *S. aureus* and *S. pneumoniae* the primary target is topoisomerase IV.<sup>8,9</sup> In Gram-positive bacteria such as *S. aureus*, the first step mutation is in *parC* and is associated with low-level resistance. The addition of a second step mutation in *gyrA* to form a *gyrA parC* double mutation is associated with high-level resistance. Conversely, in Gram-negative bacteria such as *N. gonorrhoeae* the first two mutations are in *gyrA*, the third and fourth in *parC*. The difference in quinolone target arises because gyrase from Gram-positive bacteria is less susceptible to inhibition by quinolones than gyrase from Gram-negative bacteria.

In addition, the chemical structure of the quinolone may have an effect on target preference. The primary target for ciprofloxacin in *S. pneumoniae* is topoisomerase IV, but for sparfloxacin and clinafloxacin, which have a fluorine substitution at the C-8 position, the primary target is gyrase.<sup>10</sup> These genetic studies have been elegantly supported by enzyme studies using reconstituted mutant GyrA and ParC proteins from *S. pneumoniae* bearing Ser-81→Phe and Ser-79→Phe mutations.<sup>11</sup> The proteins were overexpressed in an *E. coli* system with His-tagging and nickel chelate chromatography to yield large amounts of highly purified protein. In enzyme inhibition or DNA cleavage assays, these mutant enzyme complexes were at least eight- to 16-fold less responsive to both sparfloxacin and ciprofloxacin. The ciprofloxacin-resistant phenotype was silent in a sparfloxacin-resistant *gyrA* (Ser-81→Phe mutant) strain expressing a wild-type topoisomerase IV. In contrast, a sparfloxacin-resistant phenotype was silent in a ciprofloxacin-resistant *parC* (Ser-79→Phe mutant). This result provides strong support for sparfloxacin killing preferentially through gyrase and ciprofloxacin through topoisomerase IV, with the quinolones acting as cellular poisons rather than enzyme inhibitors.<sup>11</sup> Interestingly, a recent report suggests that moxifloxacin has DNA gyrase as its primary target in *S. aureus*, further illustrating the complexity of fluoroquinolone–target interactions.<sup>12</sup>

Computer-based studies have indicated that substitution of a fluorine or alkoxy group at the C-8 position of N-1 cyclopropyl fluoroquinolones is likely to increase their inhibitory activity against bacteria such as *S. aureus*, *Pseudomonas aeruginosa* and mycobacteria; however, quinolones containing C-8 halogens tend to exhibit unacceptable side-effects. Research has thus been focused on C-8-methoxy derivatives which exhibit low potential to cause mammalian phototoxicity and cytotoxicity whilst demonstrating good inhibitory activity against a broad spectrum of bacterial species. A study of fluoroquinolone activity against wild-type *S. aureus*

demonstrated that a C-8-methoxy substituent improved the bactericidal activity compared with a C-8-hydrogen substituent. The C-8-methoxy substituent was even more effective against a first step *parC* mutant.<sup>13</sup> This work has been extended to a wide range of C-8-substituted fluoroquinolones. It was found that C-8-methoxy-substituted compounds both blocked mutant growth and killed mutants much better than C-8-hydrogen-substituted compounds and better than those with a fluorine at C-8.<sup>14</sup> Another study, against wild-type *E. coli*, demonstrated little difference in bactericidal activity between the C-8-methoxy and C-8-hydrogen substitution. However, the bactericidal activity of the C-8-methoxy substituent was greatly improved compared with the C-8-hydrogen substituent against a first step *gyrA* mutant.<sup>15</sup> Similar results to those with *S. aureus* were seen against wild-type and *gyrA* mutants of *Mycobacterium bovis* BCG.<sup>16</sup>

Studies have shown that because of the improved bactericidal activity of C-8-methoxy substituents against resistant mutants, the likelihood of the isolation of resistant mutants is reduced.<sup>15</sup> Dense inocula (approximately  $10^{10}$  cfu) of wild-type *E. coli*, *Mycobacterium smegmatis*, *M. bovis* BCG and *Mycobacterium tuberculosis* were plated onto agar media containing C-8-methoxy substituents and C-8-hydrogen substituents. At concentrations at which no C-8-methoxy compound-resistant mutants were found, greater than  $10^3$  colonies, which were all first step *gyrA* mutants, were recovered on plates containing compounds with C-8-hydrogen substituents. *In vitro* studies with fully quinolone-susceptible strains of *S. aureus* demonstrated that first step mutants to ciprofloxacin, with a C-H substituent, appeared at a frequency of  $2.8 \times 10^{-8}$ – $3.9 \times 10^{-8}$ . The frequency of development of resistance to moxifloxacin (C-8-methoxy substituent) was much lower: 16 MRSA isolates and 15 MSSA isolates developed first step mutants with moxifloxacin at frequencies of  $1.0 \times 10^{-9}$ – $5.0 \times 10^{-9}$  and 31 isolates developed first step mutants with a frequency  $\leq 10^{-11}$ .<sup>17</sup>

All these studies used very high concentrations of quinolone. The ability of compounds to delay the development of resistance when cells are challenged with low doses has been studied in *S. aureus*.<sup>18</sup> Standard MIC determinations with two-fold dilutions were performed and cells from the highest concentration that allowed growth ( $0.5 \times \text{MIC}$ ) were passaged through another MIC determination, the MIC determinations were repeated several times in order to determine the number of passages that would occur before the population displayed high-level resistance. More passages were required for *S. aureus* to acquire resistance with moxifloxacin, a C-8 methoxy compound, than with ofloxacin, levofloxacin or trovafloxacin. These observations can be summarized by determination of the mutant prevention concentration (MPC).<sup>19</sup> MPC is determined in the same fashion as MIC except that a much larger inoculum is applied ( $10^{10}$  cfu). The antibiotic concentration at which resistant mutants are no

longer recovered is then noted, this being invariably higher than the MIC. In the case of fluoroquinolones, mutants emerge because of the selection of cells that carry mutations in both targets (*gyrA* and *parC*). The concentration required to prevent this is much lower with C-8-methoxy compounds. Thus C-8-methoxy fluoroquinolones restrict gradual step-wise selection of resistance as well as the one step acquisition of high-level resistance.

There has been some controversy over the interaction of fluoroquinolones and the detection of MRSA in patients. There is no evidence that fluoroquinolone-susceptible MRSA develop resistance to fluoroquinolones at an increased rate compared with MSSA.<sup>17</sup> The high rate of quinolone resistance in MRSA therefore seems most likely to be due to clonal expansion of a 'fit' clone by virtue of the possession of resistance to multiple antibiotics and possibly an enhanced colonization ability.

Clonal dissemination throughout the world of fluoroquinolone-resistant variants of *S. pneumoniae* has been found to be responsible for high levels of resistance in Northern Ireland<sup>20</sup> and Hong Kong.<sup>21</sup> In these cases, two molecular typing methods of DNA-BOX fingerprinting and pulsed-field gel electrophoresis (PFGE) revealed fluoroquinolone-resistant 9V and 23F in Northern Ireland and 19F, 6A and 23F in Hong Kong, to be indistinguishable from clones found originally in Spain.

### Additional mechanisms of resistance

Other mechanisms of resistance to fluoroquinolones should not be disregarded. The early findings showed that low-level resistance to quinolones relates to entry into the bacterial cell and is characterized by changes in porins, especially structural changes in OmpF and mutational changes in regulatory genes (*marA*, *soxS*, *robA*) that affect the activity of a wide range of efflux pumps.<sup>22</sup> The interplay both of changes in cell envelope preventing the ingress of antimicrobials and active efflux is complex and our view of the relative importance of both processes and the involvement of specific genes is liable to change. For instance the widely held view that the 'intrinsic' resistance of *P. aeruginosa* is due to the low permeability of the outer membrane to a wide range of antibiotics including fluoroquinolones (e.g. norfloxacin) has been challenged. Experimental evidence did not support this hypothesis.<sup>22</sup> Intrinsic levels of resistance have been found to correlate with efflux activity and even wild-type strains of *P. aeruginosa* pump out agents such as norfloxacin very effectively.<sup>23</sup> The identity of this pump is suggested by the genetic characterization that showed it to be involved in a pyoverdine (a siderophore) export operon. Mutagenic inactivation of the operon converts resistant *P. aeruginosa* into an organism that is as susceptible to antibiotics as *E. coli*.<sup>23</sup> Confusingly, mutations in the *acrA* locus lead to hypersusceptibility to quinolones and other anti-

microbials. This is because the mutation inactivates a multidrug efflux pump which is capable of pumping the dye acriflavine.<sup>24</sup> The *acrA* mutants accumulate acriflavine at an increased rate of five-fold, indicating the importance of this pump in wild strains.<sup>25</sup> Upregulation of these intrinsic efflux pumps is associated with resistance to drugs such as quinolones. Resistance is usually low level as entry is impeded but not completely prevented and clinically significant resistance is usually only seen when other resistance mechanisms are active. Recently, the crystal structure of the multidrug exporter, *acrB*, has been reported.<sup>26</sup> This provides a structural basis for considering the molecular mechanism of multidrug export, and is consistent with the possibility of a direct interaction between the multifunctional outer membrane channel, *tolC*, and *acrB*. Mechanisms such as efflux pumps may protect bacteria from low levels of antibiotics in body sites with low antibiotic levels and therefore favour the emergence of resistant mutants with higher levels of resistance (e.g. topoisomerase mutants). Mutations in the *norA* gene of *S. aureus*, which encodes a membrane-associated active efflux pump requiring norfloxacin as a substrate, have been recognized.<sup>27</sup> *norA* has recently been shown by cloning and histidine tag overexpression in *E. coli* to be a self-sufficient (i.e. not dependent on other membrane proteins) multidrug transporter pumping tetraphenyl phosphonium as well as quinolones.<sup>28</sup> Such mutations have been shown to cause moderate but clinically relevant levels of resistance.<sup>29,30</sup> A similar efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *S. pneumoniae* has been identified.<sup>31</sup> There may be other, as yet unidentified, efflux pumps that may play a role in the development of resistance to the new fluoroquinolones in both Gram-negative and Gram-positive bacteria. Evidence for the existence of an alternative reserpine-sensitive efflux pump in *S. pneumoniae* has recently been presented.<sup>32</sup> Thirty-four ciprofloxacin-resistant (MIC  $\geq 2$  mg/L) clinical isolates of *S. pneumoniae* were characterized for susceptibility and the presence of mutations in *parC/E* and *gyrA/B*. For most isolates, MICs of acriflavine and ethidium bromide were lowered by reserpine, but fluoroquinolone MICs were unaltered. Fluoroquinolone-resistant strains predominantly had mutations in three topoisomerase genes. The expression of PmrA was not exclusively associated with those isolates possessing a phenotype suggestive of the presence of an efflux pump. The clinical relevance of PmrA is therefore unclear and awaits the characterization of further efflux systems.

Bacteria belonging to the *Bacteroides fragilis* group are resistant to many agents, including older fluoroquinolones. However, newer compounds such as trovafloxacin, clinafloxacin and moxifloxacin have significant activity against them. Resistant strains with high MICs of norfloxacin have been identified and recently this resistance to norfloxacin and ciprofloxacin was ascribed to a multidrug efflux pump assigned to the recently characterized multidrug and toxic

compound extrusion (MATE) family.<sup>33</sup> The gene responsible was cloned and designated *bexA*. The *gyrA* gene of *B. fragilis* has been sequenced and was found to carry the mutation Asp-86→Phe, which is known to confer resistance to fluoroquinolones and therefore probably accounts for the intrinsic resistance of this species.<sup>34</sup>

A repair gene, *recG* in *S. aureus*, has been shown to confer low-level resistance to quinolones as insertional inactivation of the gene in a low-level resistant strain (ciprofloxacin MIC 1.56 mg/L) reduced the susceptibility to 0.2 mg/L, the same as the sensitive parental strain.<sup>35</sup> This may be important in future resistance development by again allowing selection of mutants in body sites such as mucous membranes and skin.

A report in 1998 documented a multiple resistance plasmid, pMG252 that confers resistance to aztreonam, ceftazidime, cefotaxime, cefotetan, chloramphenicol, kanamycin, gentamicin, streptomycin, sulphafurazole, tobramycin, trimethoprim and mercuric chloride and which also confers reduced susceptibility to quinolones.<sup>36</sup> It conferred only low levels of reduced susceptibility to wild-type strains, but could confer clinically significant resistance to isolates that were deficient in outer membrane proteins, particularly *Klebsiella pneumoniae*. Very recently, further information has been presented in abstract form.<sup>37</sup> The gene responsible for quinolone resistance (*qnr*) has been identified and is related to *mcbG*, which protects a microcin B17 producer from self-inhibition. The gene was found on a plasmid that, amongst other resistance genes, carried *bla*<sub>FOX-5</sub>. A survey of 275 Gram-negative bacilli failed to find *qnr* other than in isolates from Birmingham, Alabama between July 1994 and January 1995. The conclusion is that this gene is very rare and did not appear to persist.

Early work suggested that mutations in *gyrB* only led to small increases in MIC. However, as we acquire more knowledge of the interaction between topoisomerases and other resistance mechanisms, *gyrB* mutations may be more important than previously thought in resistance development. *gyrB* mutations in *S. pneumoniae* occur after *parC* and *gyrA* mutation and also after *parE* in *E. coli*.<sup>38,39</sup>

## Conclusions

With the increasing application of molecular techniques to the study of quinolone resistance, together with the availability of agents with novel structures, a better understanding of the mechanisms of action and resistance are emerging. Two major mechanisms of resistance are thought to be the most significant: mutations in the primary target (topoisomerases) and overexpression of multidrug efflux pumps. Chromosomal mutations occur in the *gyrA* and *gyrB* genes, which code for the A and B subunits of DNA gyrase and in *parC* and *parE*, which code for the C and E subunits of topoisomerase IV. The primary target for quinolones in Gram-negative

bacteria is topoisomerase II (DNA gyrase), whereas in Gram-positive bacteria, such as *S. aureus* and *S. pneumoniae*, the primary target is topoisomerase IV. The chemical structure of quinolones can have an effect on target preference and, for instance, in the case of sparfloxacin and clinafloxacin the primary target in Gram-positive bacteria is DNA gyrase. Newer quinolones such as those with a methoxy group at the C-8 position exhibit both enhanced activity against Gram-positive bacteria and anaerobes as well as a reduced rate of selection of resistance. It is believed that this is, in part, due to both DNA topoisomerase IV and DNA gyrase being targets for inhibition. Although the precise mode of action of the quinolones is not understood, it is clear that their predominant mode of action is by inhibition of DNA replication. Quinolones bind to the topoisomerase IV/DNA gyrase–DNA complexes and this results in the inhibition of DNA replication. Complex formation reversibly inhibits DNA synthesis and cell growth, which is probably responsible for the bacteriostatic action of quinolones. Their lethal action is thought to be a separate event from complex formation and probably relates to the release of free DNA ends from the DNA gyrase–quinolone complexes. Although much effort has been spent delineating and characterizing the nature of mutations in DNA topoisomerases, other mechanisms of resistance to fluoroquinolones are important. Changes in the nature and amount of porins are important and are a common mechanism conferring low-level resistance to quinolones. Decreases in expression of OmpF resulting in decreased penetration of fluoroquinolones, as well as changes in the regulatory genes *marA*, *soxS* and *roaA*, affect the activity of a wide range of efflux pumps. In Gram-positive bacteria the *norA* gene has been well characterized and, more recently, efflux pumps such as PmrA in *S. pneumoniae* have been identified and are associated with variable levels of resistance to fluoroquinolones.

Our understanding of resistance can be biased by experience. It has long been recognized that bacteria in biofilms are highly resistant to antimicrobial agents. In a recent study of *P. aeruginosa* in a biofilm model, the biofilm cells were not per se different to stationary phase planktonic cells in resistance to killing by antibiotics including ofloxacin, which can kill non-growing cells.<sup>40</sup> Persisting cells were found to be critical in the survival of both populations. The mechanism of persistence is not clear but these were not mutants, as when re-cultured they remained sensitive. Their formation does, however, require a high cell density as at low cell densities ofloxacin rapidly kills stationary phase cells.<sup>40</sup> Quorum-sensing factors may thus be involved in their production.

Quinolones may have an effect on the expression of resistance genes as opposed to co-selection of resistance to unrelated agents, the best documented being the enhanced recovery of heteroresistant oxacillin-resistant *S. aureus*.<sup>41</sup> The effect appears to be by selective inhibition or killing of the more susceptible subpopulations in heteroresistant

*S. aureus*. The surviving populations are more resistant to both oxacillin and fluoroquinolones, the mechanisms being unrelated but associated.

It might be expected that there will be a progressive increase in resistance to fluoroquinolones amongst *S. pneumoniae* with the extensive use of drugs such as ciprofloxacin, as has been seen with *P. aeruginosa* in some parts of the world. A recently published surveillance study from a range of different countries suggests that this scenario might be developing, as 0.8% of 5015 isolates of *S. pneumoniae* were found to be resistant to levofloxacin (MIC  $\geq$  8 mg/L). The highest rates were found in Hong Kong (8%), China (3.3%) and Spain (1.6%). PFGE showed that most isolates were clonal within countries, suggesting clonal spread. The most commonly encountered mutations leading to amino acid substitution were in: GyrA (Ser-81→Phe); ParC (Ser-79→Phe, Lys-137→Asn); and ParE (Ile-460→Val).<sup>42</sup> However, recent data from the USA suggests that since 1994 there has not been a major increase in resistance to fluoroquinolones in *S. pneumoniae*.<sup>43</sup> This may auger well for the preservation of the utility of the third-phase fluoroquinolones, but only time will tell.

## References

- Drlica, K. & Zhao, X. (1997). DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiology and Molecular Biology Reviews* **61**, 377–92.
- Tillotson, G. S., Dorrian, I. & Blondeau, J. (1997). Fluoroquinolone resistance: mechanisms and epidemiology. *Journal of Medical Microbiology* **46**, 457–61.
- Wiedemann, B. & Heisig, P. (1994). Mechanisms of quinolone resistance. *Infection* **22**, Suppl. 2, S73–9.
- Kato, J., Nishimura, Y., Imamura, R., Niki, H., Hiraga, S. & Suzuki, H. (1990). New topoisomerase essential for chromosome segregation in *E. coli*. *Cell* **63**, 393–404.
- Khodursky, A. B. & Cozzarelli, N. R. (1998). The mechanism of inhibition of topoisomerase IV by quinolone antibacterials. *Journal of Biological Chemistry* **273**, 27668–77.
- Zhao, X., Xu, C., Domagala, J. & Drlica, K. (1997). DNA topoisomerase targets of the fluoroquinolones: a strategy for avoiding bacterial resistance. *Proceedings of the National Academy of Sciences, USA* **94**, 13991–6.
- Chen, C. R., Malik, M., Snyder, M. & Drlica, K. (1996). DNA gyrase and topoisomerase IV on the bacterial chromosome: quinolone-induced DNA cleavage. *Journal of Molecular Biology* **258**, 627–37.
- Hoshino, K., Kitamura, A., Morrissey, I., Sato, K., Kato, J. & Ikeda, H. (1994). Comparison of inhibition of *Escherichia coli* topoisomerase IV by quinolones with DNA gyrase inhibition. *Antimicrobial Agents and Chemotherapy* **38**, 2623–7.
- Khodursky, A. B., Zechiedrich, E. L. & Cozzarelli, N. R. (1995). Topoisomerase IV is a target of quinolones in *Escherichia coli*. *Proceedings of the National Academy of Sciences, USA* **92**, 11801–5.
- Pan, X. S. & Fisher, L. M. (1997). Targeting of DNA gyrase in *Streptococcus pneumoniae* by sparfloxacin: selective targeting of gyrase or topoisomerase IV by quinolones. *Antimicrobial Agents and Chemotherapy* **41**, 471–4.
- Pan, X. S., Yague, G. & Fisher, L. M. (2001). Quinolone resistance mutations in *Streptococcus pneumoniae* GyrA and ParC proteins: mechanistic insights into quinolone action from enzymatic analysis, intracellular levels, and phenotypes of wild-type and mutant proteins. *Antimicrobial Agents and Chemotherapy* **45**, 3140–7.
- Oizumi, N., Kawabata, S., Hirao, M., Watanabe, K., Okuno, S., Fujiwara, T. *et al.* (2001). Relationship between mutations in the DNA gyrase and topoisomerase IV genes and nadifloxacin resistance in clinically isolated quinolone-resistant *Staphylococcus aureus*. *Journal of Infection and Chemotherapy* **7**, 191–4.
- Zhao, X., Wang, J. Y., Xu, C., Dong, Y., Zhou, J., Domagala, J. *et al.* (1998). Killing of *Staphylococcus aureus* by C-8-methoxy fluoroquinolones. *Antimicrobial Agents and Chemotherapy* **42**, 956–8.
- Lu, T., Zhao, X., Li, X., Drlica-Wagner, A., Wang, J. Y., Domagala, J. *et al.* (2001). Enhancement of fluoroquinolone activity by C-8 halogen and methoxy moieties: action against a gyrase resistance mutant of *Mycobacterium smegmatis* and a gyrase–topoisomerase IV double mutant of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **45**, 2703–9.
- Dong, Y., Xu, C., Zhao, X., Domagala, J. & Drlica, K. (1998). Fluoroquinolone action against mycobacteria: effects of C-8 substituents on growth, survival, and resistance. *Antimicrobial Agents and Chemotherapy* **42**, 2978–84.
- Zhao, B. Y., Pine, R., Domagala, J. & Drlica, K. (1999). Fluoroquinolone action against clinical isolates of *Mycobacterium tuberculosis*: effects of a C-8 methoxyl group on survival in liquid media and in human macrophages. *Antimicrobial Agents and Chemotherapy* **43**, 661–6.
- M'Zali, F. H., Hawkey, P. M. & Dalhoff, A. (1999). Differential selection of quinolone resistance by new quinolones in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. In *Moxifloxacin in Practice* (Adam, D. & Finch, R. G., Eds), pp. 61–9. Maxim Medical, Oxford, UK.
- Dalhoff, A. (2001). Comparative *in vitro* and *in vivo* activity of the C-8 methoxy quinolone moxifloxacin and the C-8 chlorine quinolone BAY y 3118. *Clinical Infectious Diseases* **32**, Suppl. 1, S16–22.
- Zhao, X. & Drlica, K. (2001). Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. *Clinical Infectious Diseases* **33**, Suppl. 3, S147–56.
- McGee, L., Goldsmith, C. E. & Klugman, K. P. (2002) Fluoroquinolone resistance among clinical isolates of *Streptococcus pneumoniae* belonging to international multiresistant clones. *Journal of Antimicrobial Chemotherapy* **49**, 173–6.
- Ho, P. L., Yam, W. C., Cheung, T. K., Ng, W. W., Que, T. L., Tsang, D. N. *et al.* (2001). Fluoroquinolone resistance among *Streptococcus pneumoniae* in Hong Kong linked to the Spanish 23F clone. *Emerging Infectious Diseases* **7**, 906–8.
- Nikaido, H. (1998). Antibiotic resistance caused by Gram-negative multidrug efflux pumps. *Clinical Infectious Diseases* **27**, Suppl. 1, S32–41.

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23. Li, X. Z., Livermore, D. M. & Nikaido, H. (1994). Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*: resistance to tetracycline, chloramphenicol, and norfloxacin. *Antimicrobial Agents and Chemotherapy* **38**, 1732–41.
24. Poole, K., Krebes, K., McNally, C. & Neshat, S. (1993). Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. *Journal of Bacteriology* **175**, 7363–72.
25. Ma, D., Cook, D. N., Alberti, M., Pon, N. G., Nikaido, H. & Hearst, J. E. (1993). Molecular cloning and characterization of *acrA* and *acrE* genes of *Escherichia coli*. *Journal of Bacteriology* **175**, 6299–313.
26. Murakami, S., Nakashima, R., Yamashita, E. & Yamaguchi, A. (2002). Crystal structure of bacterial multidrug efflux transporter AcrB. *Nature* **419**, 587–93.
27. Kaatz, G. W. & Seo, S. M. (1995) Inducible NorA-mediated multidrug resistance in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **39**, 2650–5.
28. Yu, J. L., Grinius, L. & Hooper, D. C. (2002). NorA functions as a multidrug efflux protein in both cytoplasmic membrane vesicles and reconstituted proteoliposomes. *Journal of Bacteriology* **184**, 1370–7.
29. Sun, L., Sreedharan, S., Plummer, K. & Fisher, L. M. (1996). NorA plasmid resistance to fluoroquinolones: role of copy number and *norA* frameshift mutations. *Antimicrobial Agents and Chemotherapy* **40**, 1665–9.
30. Yamada, H., Kurose-Hamada, S., Fukuda, Y., Mitsuyama, J., Takahata, M., Minami, S. *et al.* (1997). Quinolone susceptibility of *norA*-disrupted *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **41**, 2308–9.
31. Gill, M. J., Brenwald, N. P. & Wise, R. (1999). Identification of an efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy* **43**, 187–9.
32. Piddock, L. J., Johnson, M. M., Simjee, S. & Pumbwe, L. (2002). Expression of efflux pump gene *pmrA* in fluoroquinolone-resistant and -susceptible clinical isolates of *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy* **46**, 808–12.
33. Miyamae, S., Ueda, O., Yoshimura, F., Hwang, J., Tanaka, Y. & Nikaido, H. (2001). A MATE family multidrug efflux transporter pumps out fluoroquinolones in *Bacteroides thetaiotaomicron*. *Antimicrobial Agents and Chemotherapy* **45**, 3341–6.
34. Onodera, Y. & Sato, K. (1999). Molecular cloning of the *gyrA* and *gyrB* genes of *Bacteroides fragilis* encoding DNA gyrase. *Antimicrobial Agents and Chemotherapy* **43**, 2423–9.
35. Niga, T., Yoshida, H., Hattori, H., Nakamura, S. & Ito, H. (1997). Cloning and sequencing of a novel gene (*recG*) that affects the quinolone susceptibility of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **41**, 1770–4.
36. Martinez-Martinez, L., Pascual, A. & Jacoby, G. A. (1998). Quinolone resistance from a transferable plasmid. *Lancet* **351**, 797–9.
37. Jacoby, G. A., Chow, N. & Waites, K. (2001). Prevalence of plasmid-mediated quinolone resistance. In *Program and Abstracts of the Forty-first Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2001*. Abstract 2120, p.145. American Society for Microbiology, Washington, DC, USA.
38. Ruiz, J., Casellas, S., Jimenez de Anta, M. T. & Vila, J. (1997). The region of the *parE* gene, homologous to the quinolone-resistance determining region of the *gyrB* gene, is not linked with the acquisition of quinolone resistance in *Escherichia coli* clinical isolates. *Journal of Antimicrobial Chemotherapy* **39**, 839–40.
39. Jorgensen, J. H., Weigel, L. M., Ferraro, M. J., Swenson, J. M. & Tenover, F. C. (1999). Activities of newer fluoroquinolones against *Streptococcus pneumoniae* clinical isolates including those with mutations in the *gyrA*, *parC*, and *parE* loci. *Antimicrobial Agents and Chemotherapy* **43**, 329–34.
40. Spoering, A. L. & Lewis, K. (2001). Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *Journal of Bacteriology* **183**, 6746–51.
41. Venezia, R. A., Domaracki, B. E., Evans, A. M., Preston, K. E. & Graffunder, E. M. (2001) Selection of high-level oxacillin resistance in heteroresistant *Staphylococcus aureus* by fluoroquinolone exposure. *Journal of Antimicrobial Chemotherapy* **48**, 375–81.
42. Critchley, I. A., Blosser-Middleton, R. S., Jones, M. E., Karlowsky, J. A., Karginova, E. A., Thornsberry, C. *et al.* (2002). Phenotypic and genotypic analysis of levofloxacin-resistant clinical isolates of *Streptococcus pneumoniae* collected in 13 countries during 1999–2000. *International Journal of Antimicrobial Agents* **20**, 100–7.
43. Brueggemann, A. B., Coffman, S. L., Rhomberg, P., Huynh, H., Almer, L., Nilius, A. *et al.* (2002) Fluoroquinolone resistance in *Streptococcus pneumoniae* in United States since 1994–1995. *Antimicrobial Agents and Chemotherapy* **46**, 680–8.

