New quinolones and the impact on resistance

Thomas J. Dougherty, Danielle Beaulieu and John F. Barrett

The changes in quinolone research have been fast and exciting over the past 5–7 years with the discovery and development of several new 8-methoxy quinolones. An additional factor is the design of the so-called 4th-generation quinolones that lack the C-6 fluorine, which might impact the development of quinolone resistance. The science behind the quinolone susceptibility and resistance patterns is fascinating, but has not yet been clearly delineated in discussions of the advantages of quinolone usage in the clinic.

Thomas J. Dougherty, Danielle Beaulieu and *John F. Barrett
Department of Microbiology
Bristol-Myers Squibb Pharmaceutical Research Institute
5 Research Parkway
Wallingford, CT 06492, USA
*tel: +1 203 677 6449
fax: +1 203 677 6771
e-mail: John.Barrett@bms.com

▼ Resistance to antibiotics and antibacterials is inevitable, because these agents, by virtue of their target (i.e. bacteria), have built-in obsolescence as a result of either acquired genetic resistance (e.g. plasmid transfer, transposon) or mutational resistance[1–3]. The overall advantage of the use of quinolone agents in the clinic is the lack of a widespread transmissible resistance element that is so common with other classes of antibacterial agents (e.g. β-lactams, cephalosporins, macrolides and tetracyclines). Quinolone resistance is a result of either target modification, efflux or decreased permeability, none of which is transmissible on an extrachromosomal genetic element, to date. (However, there is a report of a resistance element on a plasmid, yet to be thoroughly characterized, which encodes a protein that binds the quinolone[4].)

Quinolone antibacterials target two topoisomerases in most pathogenic bacteria (DNA gyrase and/or topoisomerase IV). These are essential bacterial enzymes[5–17]. DNA gyrase is composed of a tetramer of two subunits each of GyrA and GyrB, and topoisomerase IV is, likewise, composed of a tetramer of two subunits each of ParC and ParE. Quinolones exhibit a wide spectrum of activity *in vitro* because these essential targets are highly conserved in virtually all bacteria. However, the diverse nature of substituents on the quinolone nucleus affords variable target activity or affinity (for DNA gyrase and/or topoisomerase IV), permeability, efflux and overall antibacterial activity[18,19].

Monitoring of resistance in the clinic

Because of its widespread use, ciprofloxacin can be used as a surrogate marker for tracking quinolone-resistance trends in the clinic. Using this surrogate, it is clear that quinolone resistance is increasing worldwide, although there are varying geographical extremes to this resistance[17,20–25]. The degree of resistance and number of pathogens affected varies from region to region, but virtually all resistance can be tracked to the antibacterial treatment paradigm in a particular clinical setting. If quinolones are used to treat gastrointestinal disorders, then quinolone resistance will probably emerge in *Escherichia coli*, *Salmonella typhimurium*, and *Shigella* spp.[17–22,24,26] upon monitoring. If quinolones are used as respiratory therapy, quinolone resistance will appear in the Gram-positive pathogens most frequently associated with respiratory diseases (e.g. *Streptococcus pneumoniae*, *Staphylococcus* spp.)[17,20,23,25].

SENTRY studies

A survey of worldwide isolates by SENTRY, an independent bacterial-isolate laboratory network, provided a three-year snapshot of the evolving scenario of resistance[21–25,27]. As part of the European network of the SENTRY Program, 25 European university hospitals provided over 9,600 blood isolates for *in vitro* testing against >20 antimicrobial agents[23]. Five pathogens accounted for two-thirds of all isolates, including *E. coli*, *Staphylococcus aureus*, coagulase-negative staphylococci, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. In this section of the SENTRY program, quinolone

1359-6446/01/$ – see front matter PII: S1359-6446(01)01760-3

resistance was detected among all Gram-negative species, and particularly in *P. aeruginosa* and *Acinetobacter* species[23].

In another study, the same isolates were tested against gatifloxacin, trovafloxacin, levofloxacin, sparfloxacin, ofloxacin and ciprofloxacin[25]. From this group, gatifloxacin and trovafloxacin exhibited the lowest minimal inhibitory concentrations (MICs) against Gram-positive cocci, including quinolone-resistant strains[25], ciprofloxacin and levofloxacin showed the lowest MIC values against *Pseudomonas* spp., whereas gatifloxacin and trovafloxacin demonstrated the highest antibacterial activity against *Acinetobacter* spp. and *Stenotrophomonas maltophilia*[25]. All *Haemophilus* spp. and *Moraxella catarrhalis* isolates were fully susceptible to all quinolones tested. Overall, the newer quinolones had improved activity compared with older quinolone agents against Gram-positive cocci and Gram-negative non-fermenters, while retaining their broad-spectrum activity against Gram-negative bacilli with low levels of resistance[25].

Bacterial pathogens (434 strains) recovered from urinary tract infections (UTIs) in hospitalized patients in Latin America were also evaluated by SENTRY[24]. *E. coli* was the most common etiological agent (60.4%), followed by *Klebsiella* spp. (11.2%), *P. aeruginosa* (8.3%) and *Enterococcus* spp. (2.3%)[24]. Less than 50% of *E. coli* isolates were susceptible to broad-spectrum penicillins, and their frequency of resistance to the more recently developed quinolones and to ciprofloxacin was also high[24]. Examination of the genetic basis for the ciprofloxacin resistance in these *E. coli* strains showed that most of the resistant isolates had a double mutation in the *gyr*A gene, as well as a single mutation in the *par*C gene. The results discussed in this review confirm that bacterial resistance, in addition to increasing quinolone resistance, continues to be a concern in Latin-American medical institutions[24].

Other studies

In a study by Thal and colleagues, the *in vitro* susceptibilities of vancomycin-resistant *Enterococcus faecium* (VRE) to ciprofloxacin, levofloxacin and clinafloxacin were examined using isolates collected between 1991 and 1996 from a regional hospital in the mid-Western USA. The MICs of 101 isolates increased every year over this six-year period, from 2 μg ml^{-1} to ≥64 μg ml^{-1}. These data indicated an alarming trend in quinolone resistance in VRE and, despite the small sample-size and possible clonal origin of these VRE strains, raised concerns for the use of quinolones as anti-enterococcal agents[a].

Kowalsky and colleagues reported the susceptibility of 2000 common respiratory isolates [*S. pneumoniae* (27%), *S. aureus* (28%), *Haemophilus influenzae* (20%), *M. catarrhalis* (7%) and *K. pneumoniae* (18%)] to moxifloxacin, in comparison to trovafloxacin, levofloxacin, penicillin, amoxicillin–clavulanate, cefuroxime, azithromycin and trimethoprim-sulfamethoxazole. MIC_{90} values for moxifloxacin ranged from 0.06 to 1 μg ml^{-1}, with moxifloxacin and trovafloxacin being the most active quinolones. However, the percentage quinolone-resistance of *S. pneumoniae* to trovafloxacin, levofloxacin and moxifloxacin was 0.9%, 1.3% and 0.5%, respectively (based on the assigned breakpoints), making moxifloxacin the most active anti-pneumococcal quinolone (in terms of MIC values) with the lowest resistance rate of the three quinolones tested[b].

Quinolone mechanisms of action

As previously mentioned, the quinolones have two bacterial enzymes as their targets: DNA gyrase and topoisomerase IV[5]. Both of these enzymes are type II DNA topoisomerases that resolve topological constraints resulting from DNA replication and function[5]. DNA gyrase is involved primarily in controlling DNA supercoiling and relieving topological stress caused by transcription and DNA replication. Topoisomerase IV functions in the intermolecular strand passage (decatenation) of daughter chromosomes after replication, as well as relaxing positively supercoiled DNA[28,29]. Both enzymes share the following reaction mechanism in common: a double-strand DNA break is introduced into duplex-DNA bound to the enzyme, a second DNA strand is passed through the break, and the DNA break is resealed and the DNA released from the enzyme[30].

Gyrase inhibition by quinolones

Quinolones have been shown to form a ternary complex with gyrase and DNA, resulting in the inhibition of DNA gyrase activities in bacteria[5–7,15,16,18,19,30]. Quinolone binding to the enzyme–DNA complex can be dissociated from quinolone-mediated DNA cleavage by DNA gyrase. Several experiments have established that DNA cleavage is not required for drug binding. Rather quinolones are believed to stabilize a conformational change that occurs in the DNA gyrase–DNA complex. This conformational change is believed to be blocked strand-passage, and shifts the cleavage-equilibrium to the cleaved form of DNA[10].

DNA gyrase isolated from resistant organisms might have changes in amino acids in either the GyrA or GyrB

[a] Thal, L.A. *et al.*, The *in vitro* susceptibilities of vancomycin-resistant *Enterococcus faecium* (VREF) to fluoroquinolones. *100th General Meeting American Society Microbiology, Los Angeles, CA, USA*

[b] Kowalsky, S. *et al.*, Moxifloxacin susceptibility program (MSP): regional and national susceptibility of common respiratory tract pathogens. *100th General Meeting American Society of Microbiology, 21–25 May, 2000, Los Angeles, CA, USA*

subunits, and in some cases, changes in both subunits can occur in a single isolate[31,32]. Changes to GyrA, which occur at amino acids in α-helices close to the active-site tyrosine, are more common. These occur along a positively charged surface that might be involved in DNA-binding, and in the region where quinolones bind to the DNA–enzyme complex. The regions in these proteins have been designated the quinolone resistance determining region (QRDR)[32], because of the common changes in key amino-acids that are repeatedly identified in resistant strains. It is unclear whether the alterations to GyrB that lead to resistance are also in proximity to the drug binding-site, although recent models suggest that this could be the case for certain conformations of the enzyme[33].

Topoisomerase IV inhibition

The role of DNA gyrase in the quinolone mechanism of action was recognized in the mid-1970s. By contrast, the role of topoisomerase IV in drug action required the later identification and appreciation of the role of the enzyme, and the finding that some quinolones had a higher affinity for topoisomerase IV than for DNA gyrase[11–13]. Mechanistically, topoisomerase IV is believed to operate similarly to DNA gyrase, as described above. Detailed studies of the interactions of quinolones with topoisomerase IV have been recently published[14–16]. This enzyme plays a crucial role in chromosome segregation by catalyzing the decatenation and relaxation of DNA *in vivo*[28]. Resistance to quinolones can also arise by point mutations that lead to changes in the amino-acid sequence of ParC and, less commonly, ParE. The affected ParC and ParE regions are similar in protein structure and in amino-acid sequence to the QRDR areas in GyrA and GyrB described above, respectively[26,32].

Dual inhibition

Most of the early inhibition studies were performed using *E. coli* gyrase and early napthyridine compounds that were forerunners to the later fluorinated quinolone compounds[30]. When topoisomerase IV was recognized as a target and purified from *E. coli*, it was found that it could also be inhibited by quinolones, albeit at higher concentrations[34,35]. Furthermore, mutants of *S. aureus* that were moderately resistant to ciprofloxacin were found to have amino acid changes in the QRDR of ParC (designated GrlA in *Staphylococcus* spp.), rather than GyrA. This suggested that the primary target for quinolones in *S. aureus* was topoisomerase IV[36]. A similar situation was observed for ciprofloxacin and *S. pneumoniae*, in that ParC had amino acid changes in moderately resistant strains. Higher resistance in both *S. pneumoniae* and *S. aureus* is associated with changes in both ParC (GrlA) and secondarily GyrA (Refs 37,38). Measurements of *in vitro* inhibition of *S. pneumoniae* enzymes (GyrAB and ParCE) with quinolones led to the unexpected finding that in *S. pneumoniae* and *S. aureus*, topoisomerase IV was more sensitive than DNA gyrase to all of the tested quinolones[38,39]. This included compounds such as sparfloxacin, that initially select for pneumococcal GyrA resistance mutations, indicating that it is this enzyme that is the primary target for this drug. It has been suggested that this discrepancy between biochemical measurements of drug inhibition and the genetic selection of resistant mutants could reflect the differential lethality of cleavable complexes in the intact bacteria, based on which killing pathway is most important[40].

Quinolone lethality

The initial experiments looking at quinolone lethality indicated that quinolones rapidly and reversibly inhibited DNA synthesis in bacteria[6,18,19]. Additional evidence leads to the conclusion that inhibition of DNA synthesis in itself is not the lethal event in quinolone-induced killing. The interaction of quinolone compounds with bacteria leads to the formation of a quinolone–enzyme–DNA complex, with either gyrase or topoisomerase IV[41] or both. The finding that complex formation was reversible[5,41], led to the conclusion that this was not the lethal event either. It is now believed that cell death results from the release of double-stranded DNA breaks from the drug–enzyme–DNA complex[5,6,41]. It is currently postulated that quinolone treatment might also induce the expression of a protein that participates in the release of the topoisomerase (gyrase or topoisomerase IV) from the ternary complex, leading to the release of DNA with a double-strand break[41]. Thus, it is believed that there are two steps to quinolone action: formation of bacteriostatic drug–enzyme–DNA complexes, followed by the release of lethal double-stranded DNA breaks.

Quinolone resistance mechanisms

Resistance mechanisms, in general, vary from species to species, but all can be grouped into six major classes: (1) target modification by an exogenous factor; (2) target mutation; (3) drug modification or hydrolysis; (4) efflux; (5) loss of permeability; and (6) a novel plasmid-inactivation mechanism. Of these six, target modification and plasmid inactivation are the major mechanisms of quinolone resistance[17–19,26,42].

Target spontaneous mutation

The selection of bacterial mutant clones in which spontaneous mutations have occurred *in vitro* or *in vivo*, and the subsequent selection of 'resistant' mutants in the presence of an antibacterial agent, such as a quinolone, has been well

documented, and for virtually every class of antibiotic used worldwide[1–3,17]. As described above, drug-target mutations occur in both DNA gyrase and topoisomerase IV, leading to stepwise increases in the resistance to quinolone compounds[26,32], as measured by MIC values. These spontaneous mutations are selected and accumulate upon quinolone exposure, ultimately leading to pathogens with multiple target-mutations and high-level quinolone resistance.

Efflux

Efflux is one of the two mechanisms by which bacteria become resistant that are not related to the drug target *per se* (the other being permeability), but occur by reducing access of the drug to the target[42–45]. Efflux pumps are ubiquitous in both Gram-positive and Gram-negative bacteria; they efficiently rid the cell of potentially toxic products encountered in everyday bacterial environments[17,42,43]. Unfortunately, many classes of antibiotics, including quinolones, are subject to efflux. In *S. aureus*, the efflux pump NorA is responsible for decreased susceptibility to fluoroquinolones, which has resulted in their limited use in some clinical settings[17,26,42,45]. Hydrophilic quinolones such as norfloxacin, ciprofloxacin and ofloxacin are effectively pumped by this system, but newer, hydrophobic quinolones, such as sparfloxacin, trovafloxacin and moxifloxacin, are not as affected by NorA[43,44,46–48].

Resistance because of increased expression of efflux pumps usually leads to low-level resistance, but it also increases the potential for high-level resistance. Fluoroquinolone resistance in clinical isolates is usually attributable to target mutations (as described previously), but it has been reported that many of these strains also over-express NorA, or at least have decreased resistance when the efflux-pump blocker, reserpine, is used[44,46]. For NorA, the increased expression comes from a mutation in the putative promoter region[44,49]. Additionally, resistance is also attributed to a mutation in the *norA* structural gene itself[44,49].

A gene similar to *norA*, named *pmrA*, has been identified in *S. pneumoniae*. Although clinical resistance to quinolones in *S. pneumoniae* is still low, it has been shown that 33–51% of those resistant strains demonstrate enhanced efflux properties, as determined using reserpine, which suggests efflux might contribute to the development of resistance in this species[c].

[c] Critchely, I. *et al.*, Activity of moxifloxacin (MFX) against resistant and susceptible populations *of Streptococcus pneumoniae* (SP), *Haemophilus influenzae* (HI), and *Moraxella catarrhalis* (MC) isolated in the United States. *39th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 26–29 September 1999, San Francisco, CA, USA*

Although laboratory efflux-mutants have been identified that have a wide range of MIC values greater (2–16 times) than that of the wild-type strain, the general belief is that efflux is responsible for a small alteration in the susceptibility of quinolones, as measured by MIC values[43]. The most probable major contribution to resistance is the lowering of the actual target-accessible concentration of drug at the target site, thus increasing the likelihood of further selection of resistance by the target-mutation mechanisms[43].

Other resistance mechanisms

There has been a report of plasmid-mediated quinolone resistance in *K. pneumoniae*[4] that is attributable to a protein which sequesters the quinolone in a complex, preventing interaction with the target proteins. Further investigations of this interesting mechanism are warranted.

Developments in novel quinolones

8-Methoxy quinolones

The two latest additions to the quinolone arsenal of antibacterials to reach the market are moxifloxacin and gatifloxacin, which were both approved for sale in December 1999 and contain an 8-methoxy group (Fig. 1).

Moxifloxacin demonstrates excellent activity against *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*[c,47,50]. It is also effective against Gram-positive bacteria, including those resistant to other earlier generation quinolone antibiotics[47,51]. Moxifloxacin has been well-studied in terms of mechanism-of-action, and inhibits both DNA gyrase and topoisomerase IV (Ref. 52). Further, it was reported that multi-step resistance against moxifloxacin developed more slowly in comparison to other quinolones[52].

In one study, the resistance mutation rate of moxifloxacin (10^{-9}), was less than the 10^{-6}–10^{-7} range at $4 \times$ MIC that is typically encountered[51]. Moxifloxacin also retains high activity against first- and second-step ciprofloxacin- and ofloxacin-resistant mutants, which indicates a potential clinical value for infections caused by quinolone-resistant organisms[51].

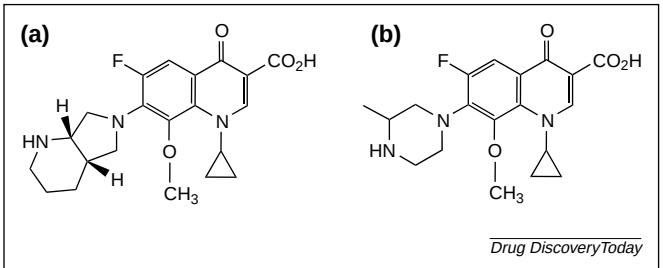


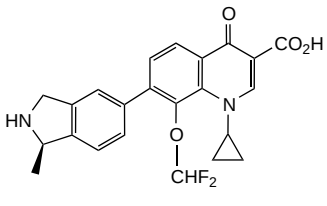
Figure 1. (a) Moxifloxacin and **(b)** gatifloxacin.

Another 8-methoxy quinolone agent, gatifloxacin, is a broad-spectrum quinolone that has excellent activity against Gram-positive bacteria[d,e,21–25,53] such as the ciprofloxacin-resistant strains *S. pneumoniae*, and *S. aureus*[d,e]. Work by Drlica and colleagues has indicated a strong structure–activity relationship (SAR) for the 8-methoxy group on the quinolone nucleus as a major factor in the reduced selection of resistant strains of bacteria[6,53]. The 8-methoxy group makes the compound more active against GyrA with resistance mutations in the α-helix 4 region[53], which is the area associated with quinolone binding. These data indicated that the 8-methoxy group also enhanced activity against topoisomerase IV. The 8-methoxy group enhanced activity by both growth inhibition and quinolone lethality. When lethal effects were measured (at a constant multiple of the MIC for each fluoroquinolone to normalize for differences in bacteriostatic action), gatifloxacin was more potent compared with non-8-methoxy compounds[53]. Taken together, these data suggest that the 8-methoxy group might restrict the selection of quinolone-resistant mutants because of its enhanced ability to block mutant growth and kill mutant cells[6,53].

Des-*quinolones*

BMS-284756 is a novel gyrase inhibitor lacking the C-6 fluorine typically present on quinolones[54]. The compound contains an 8-difluoro-methoxy group (Fig. 2). In one study, BMS-284756 was either equally or more active (i.e. reduced MIC values) against Gram-positive pathogens than all other quinolones tested (with the exception of *E. faecium*), with reported MIC_{90} values of 0.03–2 μg ml^{-1}. BMS-284756 had the highest *in vitro* activity against ciprofloxacin-resistant *S. pneumoniae*, with an MIC_{90} value of 0.5 μg ml^{-1}, compared with trovafloxacin, moxifloxacin, levofloxacin, ofloxacin and ciprofloxacin that had MIC_{90} values of 4, 2, 8, 16 and 16 μg ml^{-1}, respectively[f].

BMS-284756 was effective against all of 200 ciprofloxacin-resistant *S. pneumoniae* isolates, at or below the anticipated breakpoint (based on pharmacokinetic data) of 4 μg ml^{-1}; indeed, these isolates showed 100% susceptibility at 2 μg ml^{-1}. Similarly, in a SENTRY study of almost 25,000 isolates from Europe and the USA collected in 1999, of which 11.3% of the isolates (955) were *S. pneumoniae*, the MIC_{90} values for BMS-284756, ciprofloxacin, and trovafloxacin were 0.12, 2 and 0.25 μg ml^{-1}, respectively[g].



Drug DiscoveryToday

Figure 2. BMS-284756 (T-3811)

In examining the frequency of resistance emergence, site of mutation and degree of cross-resistance between BMS-284756, ciprofloxacin, moxifloxacin and levofloxacin, Hartman-Neumann and coworkers reported the selection and characterization of mutants of *S. pneumoniae*, selected using either BMS-284756 or ciprofloxacin. Selection with BMS-284756 yielded stepwise mutants of *S. pneumoniae* (at a rate of 1 in 10^{-9} at 4 × the MIC), with MIC values from 0.015 μg ml^{-1} (parental strain) to 8–16 μg ml^{-1} for BMS-284756, in four discrete steps (Table 1). The resistance of the other quinolones compared in this study also rose; however, ciprofloxacin, levofloxacin and moxifloxacin all had MIC values above the resistance cut-off point at the second of the four levels selected (Table 1). By contrast, for ciprofloxacin-selected mutants (at a rate of 1 in 10^{-7} at 4 × the MIC) MIC values increased from 0.5 μg ml^{-1} (parent) to 16 μg ml^{-1} in two resistance steps (Table 2). Levofloxacin had a similar increase to 16 μg ml^{-1}, and moxifloxacin had an MIC value of 2 μg ml^{-1} by the second step, whereas BMS-284756 MIC values were only 0.5 μg ml^{-1} at the second step. This indicates that with ciprofloxacin-selected mutants, ciprofloxacin loses 'clinical' susceptibility by the first-step mutation, levofloxacin and moxifloxacin by the second-step mutation, but BMS-284756 maintains susceptibility into (if not beyond) the second step. Thus, ciprofloxacin-resistant laboratory mutants are still inhibited by BMS-284756, suggesting that BMS-284756 could potentially be used to cover first- and second-step quinolone-resistant mutants in the clinic, based on this data and the antibiotic levels observed in early human pharmacology dosing studies[h]. It is now necessary to determine if these *in vitro* generated results can be reproduced in the clinic.

[d] Thomson, K.S. *et al.*, Activity of gatifloxacin against clinical isolates with various levels of resistance to ciprofloxacin. *39th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 26–29 September 1999, San Francisco, CA, USA*

[e] Kishii, R. *et al.*, Bactericidal activity of gatifloxacin, a new 8-methoxy quinolone, against quinolone-resistant *Staphylococcus aureus*. *39th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 26–29 September 1999, San Francisco, CA, USA*

[f] Gradelski, E. *et al.*, The *in vitro* activity of the novel *des*-(6) fluoro quinolone BMS-284756 against Gram-positive and Gram-negative aerobic bacteria. *40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

[g] Jones, R.N. *et al.*, BMS-284756 (BMS) activity and spectrum tested against pathogens isolated in the United States (US) and Europe (EU): Report from the SENTRY antimicrobial surveillance program (1999). *40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

[h] Hartman-Neumann, S.L. *et al.*, Selection and characterization of BMS-284756 resistant mutants in *Streptococcus pneumoniae*. *40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

Table 1. Minimal inhibitor concentrations (μg ml^{-1}) of quinolones against BMS-284756 resistant mutants of *Streptococcus pneumoniae* R6

Strain	BMS-284756	Ciprofloxacin	Levofloxacin	Moxifloxacin
R6 parent	0.015	0.5	0.5	0.125
1st level	0.06	1.0	1.0	0.25
2nd level	0.25	16.0	16.0	4.0
3rd level	2.0	16.0	16.0	4.0
4th level	16.0	16.0	64.0	64.0

Table 2. Minimal inhibitor concentrations (μg ml^{-1}) of key quinolone antibiotics against ciprofloxacin-resistant mutants of *Streptococcus pneumoniae* R6

Strain	Ciprofloxacin	BMS-284756	Levofloxacin	Moxifloxacin
R6 parent	0.5	0.015	0.5	0.125
1st level	2.0	0.06	2.0	0.25
2nd level	16.0	0.5	16.0	2.0

Non-fluorinated quinolones

Quinolone derivatives that completely lack fluorine have also been reported (non-fluorinated quinolones or NFQs). The purpose of the strategy to eliminate fluorine substituents has been to design new-generation quinolones without the same class-related toxicities of existing 6-fluoroquinolones[i,j]. These NFQs have shown good *in vitro* activities against clinical pathogens, with overall *in vitro* antibacterial activities matching those of their fluorinated counterparts[k–p]. The NFQs target both quinolone targets (DNA gyrase and topoisomerase IV)[n]. Interestingly, these NFQs maintain *in vitro* activity in the presence of many of the frequently reported QRDR mutations, and appear to select unique resistance-mutations outside the QRDR[o]. In addition to targeting DNA synthesis, the NFQs apparently also inhibit RNA synthesis[p].

Conclusions

Increased isolation of antibiotic resistant bacteria is a normal outcome of the process of antibiotic usage in the clinic. Spontaneous mutations occur in all bacteria, and selection of resistant survivors, under insult from an external agent (i.e. antibiotics) occurs at rates that typically vary from 1×10^{-5} to 1×10^{-12} (Refs 18,19). Antibiotics themselves do not cause resistance; the spontaneously arising resistant mutants are selected and amplified in the presence of antibiotics. In the absence of antibiotic use, there would be no widespread amplification and dissemination of antibiotic resistance.

[i] Gray, J.L. *et al.*, Synthesis and testing of non-fluorinated quinolones (NFQs), A study on the influence of the C6 position. *40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

[j] Catrenich, C.E. *et al.*, Non-fluorinated quinolones (NFQs): unique properties and potential opportunities for the treatment of resistant pathogens. *40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

[k] Felmingham, D. *et al.*, *In vitro* activity of non-fluorinated quinolones (PGE-9262932, -4175997, -9509924) against *Mycoplasma pneumoniae, Chlamydiae pneumoniae* and *Legionella* spp. *40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

[l] Sahm, D.F. *et al.*, Activities of non-fluorinated quinolones against recent clinical isolates of gram-positive cocci, including those resistant to currently available fluoroquinolones. *40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

[m] Brown, S.D. *et al.*, *In vitro* activities of 3 non-fluorinated quinolones and 3 fluroquinolones against consecutive clinical isolates from eleven U.S. medical centers. *40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

[n] Roychoudhury, S. *et al.*, Whole cell inhibition of dual molecular targets by a series of nonfluoroquinolones (NFQs) in *Staphylococcus aureus. 39th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 26–29 September 1999, San Francisco, CA, USA*

[o] Roychoudhury, S. *et al.*, Isolation and analysis of novel *Staphylococcus aureus* mutants via exposure to subinhibitory levels of non-fluorinated quinolones (NFQs) in a serial passage experiment. *40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

[p] Renick, P.J. *et al.*, Metabolic specificity profiling of fluoroquinolones, non-fluorinated quinolones, and other DNA topoisomerase inhibitors against intact cells of *Staphylococcus aureus. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 17–20 September 2000, Toronto, Ontario, Canada*

In many Gram-negative pathogens, moderate-level resistance to early-generation quinolone compounds (e.g. ciprofloxacin, norfloxacin) arises from a mutation in the GyrA protein, and high-level resistance arises from mutation of a second GyrA and/or topoisomerase IV site[5]. For some Gram-positive bacteria, the primary target is reversed from what is normally found in *E. coli* and other Gram-positive microorganisms. Instead of DNA gyrase being the primary target, resistance occurs as a result of changes in topoisomerase IV, whereas alterations in gyrase provide additional resistance[5]. The design of compounds with a 'balanced' activity and affinity against both primary topoisomerase targets (DNA gyrase and topoisomerase IV), makes the selection of concomitant genetic resistance to both targets and, therefore, emergence of resistance, less likely[6,18,32]. In summary, quinolones such as ciprofloxacin and levofloxacin, without a balance of target activity, might risk a higher rate of resistance emergence, whereas balanced, dual-target agents such as gatifloxacin and moxifloxacin should have a lower rate of resistance emergence *in vitro*. The concept of Mutant Prevention Concentrations (MPCs) is thought to be an important factor in determining whether a particular quinolone will select resistance[55–57]. This measure evaluates the ability of a quinolone compound to block the growth of first-step resistance mutants without regard to the mechanism of resistance. The concept is that a compound that has a similar susceptibility (MIC) for both parent and first-step mutants would require two simultaneous resistance mutations to acquire significant resistance and survive, which is an unlikely occurrence[57].

Numerous reports have documented the selection of primary and secondary quinolone-resistant mutants; those with high-level resistance have one or more target (DNA gyrase and/or topoisomerase IV) mutations responsible for the increase in MIC values. Because there is no known plasmid-mediated resistance factor disseminating quinolone resistance, and no known enzymic hydrolyzing or modifying resistance-factor, quinolone resistance will probably continue to increase as a result of the selection of spontaneous resistance mutants in the clinic, and/or the continued selection of pre-existing low level resistance strains that are unsuccessfully treated. At this time, widespread transmissible quinolone resistance, as with β-lactamases, is unlikely to occur quickly.

The use of more potent quinolone agents, such as the *des*-fluoroquinolone BMS-284756, as well as the use of 8-methoxy quinolones such as moxifloxacin and gatifloxacin, could reduce the occurrence and dissemination of quinolone-resistant Gram-positive pathogens. Extensive monitoring of susceptibility and genotyping of the resistant strains by surveillance programs such as SENTRY will be needed to confirm this hypothesis. Equally important is the appropriate use of quinolones in general, considering *in vitro* activity, *in vivo* potency (based on biophysical parameters of the compounds and pharmacodynamic parameters) and safety factors. Not all quinolones are created equal, and the design of new quinolones that either suppress resistance or inhibit quinolone-resistant strains of bacteria will probably be the most successful approach for the next generation of quinolones.

References

1 Breithaupt, H. (1999) The new antibiotics. *Nat. Biotechnol.* 17, 1165–1169
2 Wise, R. (1999) A review of mechanisms of action and resistance of antimicrobial agents. *Can. Respir. J.* 6 (Suppl. A), 20A–22A
3 Stefani, S. and Agodi, A. (2000) Molecular epidemiology of antibiotic resistance. *Int. J. Antimicrob. Agents* 13, 143–153
4 Martinez, L. *et al.* (1998) Quinolone resistance from a transferable plasmid. *Lancet* 351, 797–799
5 Drlica, K. and Zhao, R. (1997) DNA gyrase, topoisomerase IV and the 4-quinolones. *Microbiol. Mol. Biol. Revs.* 61, 377–392
6 Drlica, K. (1999) Mechanism of fluoroquinolone action. *Curr. Opin. Microbiol.* 2, 504–508
7 Reece, R. and Maxwell, A. (1991) DNA gyrase: structure and function. *Crit. Rev. Biochem. Mol. Biol.* 26, 335–375
8 Sugino, A. *et al.* (1977) Mechanism of action of nalidixic acid: purification of *Escherichia coli nalA* gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. *Proc. Natl. Acad. Sci. U. S. A.* 74, 4767–4771
9 Gellert, M. *et al.* (1977) Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. *Proc. Natl. Acad. Sci. U. S. A.* 74, 4772–4776
10 Kampranis, S.C. and Maxwell, A. (1998) The DNA gyrase–quinolone complex: ATP hydrolysis and the mechanism of DNA cleavage. *J. Biol. Chem.* 273, 22615–22626
11 Kato, J-L. *et al.* (1990) New topoisomerase essential for chromosome segregation in *E. coli*. *Cell* 63, 393–404
12 Khodursky, A.B. *et al.* (1995) Topoisomerase IV is a target of quinolones in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 92, 11801–11805
13 Blanche, R. *et al.* (1996) Differential behaviors of *Staphylococcus aureus* and *Escherichia coli* type II DNA topoisomerases. *Antimicrob. Agents Chemother.* 40, 2714–2720
14 Marians, K.J. and Hiasa, H. (1997) Mechanism of quinolone action. A drug-induced structural perturbation of the DNA precedes strand cleavage by topoisomerase IV. *J. Biol. Chem.* 272, 9401–9409
15 Anderson, V.E. *et al.* (1998) Topoisomerase catalysis and the mechanism of quinolone action. *J. Biol. Chem.* 273, 17879–17885
16 Khodursky, A.B. and Cozzarelli, N.R. (1998) The mechanism of inhibition of topoisomerase IV by quinolone antibacterials. *J. Biol. Chem.* 273, 27668–27677
17 Piddock, L.J.V. (1999) Mechanism of fluoroquinolone resistance: An update 1994–1998. *Drugs* 58 (Suppl. 2), 11–18
18 Hooper, D.C. and Wolfson, J.S., eds (1993) *Quinolone Antimicrobial Agents* (2nd edition), American Society for Microbiology, Washington DC
19 Andriole, V.T., ed. (1998) *The Quinolones* (2nd edition) Academic Press
20 Goldstein, F.W. and Acar, J.F. (1995) Epidemiology of quinolone resistance: Europe and North and South America. *Drugs* 49 (Suppl. 2), 36–42
21 Fluit, A.C. *et al.* (1997) Antimicrobial resistance among urinary tract infection (UTI) isolates in Europe: Results from the SENTRY Antimicrobial Surveillance Program. *Antonie van Leeuwenhoek Int. J.* 77, 147–152

22 Diekema, D.J. *et al.* (1997) Survey of bloodstream infections due to gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada and Latin America for the SENTRY Antimicrobial Surveillance Program, 1997. *Clin. Infect. Dis.* 29, 595–607

23 Fluit, A.C. *et al.* (2000) Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY antimicrobial surveillance program, 1997 and 1998. *Clin. Infect. Dis.* 30, 454–460

24 Gales, A.C. *et al.* (2000) Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial surveillance program, 1998. *J. Antimicrob. Chemother.* 45, 295–303

25 Schmitz, F.J. *et al.* (1999) Comparative activities of six different fluoroquinolones against 9,682 clinical bacterial isolates from 20 European university hospitals participating in the European SENTRY surveillance programme. The SENTRY participants group. *Int. J. Antimicrob. Agents* 12, 311–317

26 Hooper, D.C. (1999) Mechanisms of quinolone resistance. *Drug Resist. Updates* 2, 38–55

27 Jones, R.N. (2000) Perspectives on the development of new antimicrobial agents for resistant Gram-positive pathogens. *Braz. J. Infect. Dis.* 4, 1–8

28 Adams, D. *et al.* (1992) The role of topoisomerase IV in partitioning bacterial replicons and the structure of catenated intermediates in DNA replication. *Cell* 71, 277–288

29 Crisona, N.J. *et al.* (2000) Preferential relaxation of positively supercoiled DNA by *E. coli* topoisomerase IV in single-molecule and ensemble measurements. *Genes Dev.* 14, 2881–2892

30 Roca, J. (1995) The mechanisms of DNA topoisomerases. *Trends Biochem. Sci.* 20, 156–160

31 Nakamura, S. *et al.* (1989) *GyrA* and *gyrB* mutations in quinolone-resistant strains of *Escherichia coli*. *Antimicrob. Agents Chemother.* 33, 254–255

32 Nakamura S. (1997) Mechanisms of quinolone resistance. *J. Infect. Chemother.* 3, 128–138

33 Fass, D. *et al.* (1999) Quaternary changes in topoisomerase II may direct orthogonal movement of two DNA strands. *Nat. Struct. Biol.* 6, 322–326

34 Peng, H. and Marians, K. (1995) The interaction of *Escherichia coli* topoisomerase IV with DNA. *J. Biol. Chem.* 270, 25286–25290

35 Hoshino, K. *et al.* (1994) Comparison of inhibition of *Escherichia coli* topoisomerase IV by quinolones with DNA gyrase inhibition. *Antimicrob. Agents Chemother.* 38, 2623–2627

36 Ferrero, L. *et al.* (1994) Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: a primary target of fluoroquinolones. *Mol. Microbiol.* 13, 641–653

37 Munoz, R. and de la Campa A.G. (1996) ParC subunit of DNA topoisomerase IV of *Streptococcus pneumoniae* is a primary target of fluoroquinolones and cooperates with DNA gyrase A subunit in forming resistance phenotype. *Antimicrob. Agents Chemother.* 40, 2252–2257

38 Pan, X-S. *et al.* (1996) Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 40, 2321–2326

39 Pan, X-S. and Fisher, L.M. (1998) DNA gyrase and topoisomerase IV are dual targets of clinafloxacin action in *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 42, 2810–2816

40 Pan, X-S. and Fisher, L.M. (1999) *Streptococcus pneumoniae* DNA gyrase and topoisomerase IV: Overexpression, purification and differential inhibition by fluoroquinolones. *Antimicrob. Agents Chemother.* 43, 1129–1136

41 Chen, C-R. *et al.* (1996) DNA gyrase and topoisomerase IV on the bacterial chromosome: quinolone-induced DNA cleavage. *J. Mol. Biol.* 258, 627–637

42 Rattan, A. (1999) Mechanisms of resistance to fluoroquinolones. *Natl. Med. J. India* 12, 162–164

43 Lawrence, L.E. and Barrett, J.F. (2000) Inhibition of bacterial efflux: needs, opportunities and strategies. *Current Opin. Anti-Infective Invest. Drugs* 2, 145–153

44 Ng, E.Y.W. *et al.* (1994) Quinolone resistance mediated by NorA: physiologic characterization and relationship to flqB, a quinolone resistance locus on the *Staphylococcus aureus* chromosome. *Antimicrob. Agents Chemother.* 38, 1345–1355

45 Hooper, D.C. (1998) Clinical applications of quinolones. *Biochim. Biophys. Acta* 1400, 45–61

46 Markham, P.N. *et al.* (1999) Multiple novel inhibitors of the NorA multidrug transporter of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 43, 2404–2408

47 Alcala, L. *et al.* (1999) *In vitro* activity of the new quinolone moxifloxacin (Bay128039) against resistant Gram-positive clinical isolates. *Diagn. Microbiol. Infect. Dis.* 33, 27–31

48 Pestova, E. *et al.* (2000) Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones. *J. Antimicrob. Chemother.* 45, 583–590

49 Lee, A. *et al.* (2000) Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. *J. Bacteriol.* 182, 3142–3150

50 Gill, M.J. *et al.* (1999) Identification of an efflux pump gene, pmrA, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 43, 187–189

51 Pong, A. *et al.* (1999) Activity of moxifloxacin against pathogens with decreased susceptibility to ciprofloxacin. *J. Antimicrob. Chemother.* 44, 621–627

52 Dalhoff, A. *et al.* (1996) *In vitro* activity of BAY128039, a new methoxyquinolone. *Chemotherapy* 42, 410–425

53 Lu, T. *et al.* (1999) Gatifloxacin activity against quinolone-resistant gyrase: allele-specific enhancement of bacteriostatic and bactericidal activities by the C-8-methoxy group. *Antimicrob. Agents Chemother.* 43, 2969–2974

54 Takahata, M. *et al.* (1999) *In vitro* and *in vivo* antimicrobial activities of T3811, a novel *des*-F(6)-quinolone. *Antimicrob. Agents Chemother.* 43, 1077–1084

55 Blondeau, J.M. *et al.* (2001) Mutant prevention concentrations of fluoroquinolones for clinical isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother.* 45, 433–483

56 Sindelar G. *et al.* (1999) Mutant prevention concentration as a measure of fluoroquinolone potency against mycobacteria. *Antimicrob Agents Chemother.* 44, 3337–3343

57 Zhao X. *et al.* (1997) DNA topoisomerase targets of the fluoroquinolones: a strategy for avoiding bacterial resistance. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13991–13996

Scirus – new science search engine launched

Elsevier Science has announced the launch of its new science search engine, Scirus, a part of the ScienceDirect family of products. Using the latest in search-engine technology, developed by Fast Search and Transfer (FAST), Scirus is able to locate both free and access-controlled scientific information across the entire World Wide Web that conventional search engines miss. Scirus will cover Elsevier Science's leading research resources: ChemWeb, BioMedNet and ScienceDirect, and the company is in negotiations with other publishers to allow Scirus to search their proprietary databases.