Inhibitors of Bacterial Topoisomerases:
Mechanisms of Action and Resistance and Clinical Aspects[1]

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Abstract: The quinolone class of inhibitors of bacterial type II topoisomerases has gained major clinical importance during the last years due to improvements in both pharmacokinetic and pharmacodynamic properties. These include favorable bioavailability allowing oral administration, good tolerability, high tissue concentrations as well as superior bactericidal activity against a broad spectrum of clinically relevant pathogens, like enterobacteria, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. In addition, no enzymatic mechanism of drug inactivation exists in bacteria and no indications for transfer of clinically relevant resistance exist. Nevertheless, resistance is being increasingly reported, even for naturally highly susceptible species like *Escherichia coli*. The underlying mechanisms of resistance include alterations in both bacterial targets, DNA gyrase and topoisomerase IV, often combined with mutations affecting drug accumulation, e.g., by increased drug efflux, reduced drug influx, or both. Investigations aiming at understanding the molecular mechanisms of quinolone action and resistance in more detail should provide a basis for a rational design of more potent derivatives. In addition, a prudent use of these highly valuable "magic bullets" is necessary to preserve their potential for the future.

Key words: Bacterial topoisomerase, fluoroquinolone, bacteria and antibiotic resistance.

Introduction

According to the World Health Report on Infectious Diseases 2000 overcoming antibiotic resistance is the major issue of the WHO for the next millenium. Currently, antibiotics provide the main basis for a causative therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new, more potent antibiotics.

During the last decades a limited number of well established classes of antibiotics, e.g., β-lactams, macrolides or quinolones, have been investigated extensively to identify the mechanism of action and the molecular structure of the target site. Based upon this knowledge, new antibiotics were designed by chemical modification of approved prototype compounds. However, this led to a limitation of therapeutic options for those bacteria which have already developed high resistance to these prototypes.

In contrast, the novel genomics approach uses DNA sequence data bases to identify novel lethal targets. However, sophisticated systems for high-throughput screening of chemical and biological molecule libraries are needed in order to identify lead compounds, which subsequently require additional research capacities for elucidating the underlying mechanism of action (1).

Starting with a well characterized target structure to screen for alternate, chemically unrelated inhibitors seems to be another promising approach. Thus, bacterial type II topoisomerases can be considered as targets not only for known but also for potential novel antibiotics.

DNA Toposiomerases

Topoisomerases play an essential role for the control of the three-dimensional DNA structure in all cells (eubacteria, eukaryote, archaea). According to their mechanism of action topoisomerases are classified as type I or type II enzymes [for a recent review see (2)].

The first topoisomerase detected was the bacterial type I enzyme, topoisomerase I (3), which in cooperation with DNA gyrase, a bacterial type II enzyme, enzymatically controls the maintenance of the DNA supercoiling degree (4). Among all topoisomerases DNA gyrase is unique by its ability to introduce so-called negative supercoils into covalently closed circular (ccc) double stranded DNA in the relaxed state (Fig. 1).

DNA gyrase holoenzyme consists of two pairs of subunits, GyrA and GyrB, the products of the corresponding genes *gyrA* and *gyrB*, respectively, and forms a tetrameric structure (A_2B_2) (Fig. 2). Both subunits contain functional domains involved in interactions with DNA, with ATP (ATP-binding motif in GyrB), and between subunits A and B [for a review see ref. (5)] Fig. 2.

[1] Experimental work was performed at the Department of Pharmaceutical Microbiology at the University of Bonn
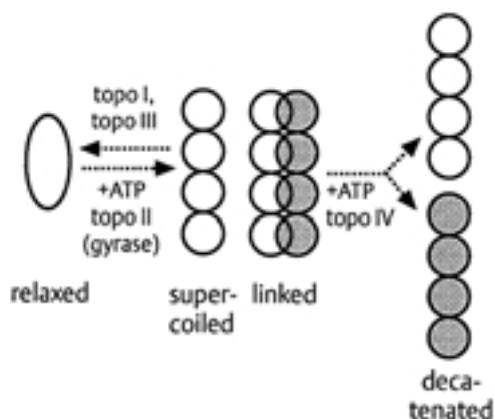
Fig. 1 Biological functions of bacterial topoisomerases. In the relaxed state of a covalently closed circular DNA in the B conformation the helical axis is planar. Every 10.4 base pairs (bp) one DNA strand its wound around the other forming one helical turn and, simultaneously, the two strands are topologically linked like links of a chain. Thus, in the relaxed state the number of links equals the number of turns and the helical axis is planar. At the expense of ATP hydrolysis topoisomerase II (DNA gyrase) converts a ccc DNA from the relaxed to the supercoiled state. This poses torsional stress to the DNA molecule, which is relieved by writhing the helical axis into the space (supercoiled form). In one reaction cycle (Fig. 2) DNA gyrase reduces the number of topological links between the DNA strands in steps of two (type II topoisomerase) while maintaining the number of helical turns constant according to the B conformation. Thus, by definition, two negative supercoils are introduced, i.e., the DNA molcule is "underwound". (Increasing the number of topological links yields positive supercoils). Negatively supercoiled DNA has a higher content of energy, which is exploited to facilitate local unwinding of, e.g., promoter regions and to drive polymerization reactions along the DNA strands (DNA replication, transcription). To maintain a moderate level of negative supercoiling topoisomerase I and, under certain circumstances (i.e., recombinational repair events), topoisomerase III catalyze relaxation of negatively supercoiled DNA. Topoisomerase IV is another type II toposiomerase capable of separating daughter chromosomes at the end of a replication cycle before cell division occurs. Topo IV reaction also consumes ATP (5).

The current model explaining the molecular mechanism of action of type II topoisomerases is based upon biochemical and genetic investigations of DNA gyrase from *E. coli* and crystallographic analysis of a homologous type II topoisomerase from *Saccharomyces cerevisiae* (6), (7). This model postulates a multi-step mechanism schematically outlined in Fig. 2.

Recently, another bacterial type II topoisomerase, topoisomerase IV (topo IV), has been identified in *E. coli* (8). Both enzymes – gyrase and topo IV – share several structural and functional features. Based upon protein sequence homology to DNA gyrase, analogous domains (e.g., ATP-binding) are assigned to subunits A (ParC) and B (ParE) of topo IV. In contrast to DNA gyrase, topo IV catalyzes the separation (decatenation) of two double-stranded ccc DNA molecules intertwined like links of a chain (Fig. 1). This is a prerequisite for the termination of the DNA replication and the subsequent cell division (9).

While the gyrase-mediated reaction involves intramolecular strand passage (the T-segment belongs to the same DNA molecule as the G-segment, Fig. 2) topo IV favors an intermolecular strand transfer (T-segment is part of a second ccc
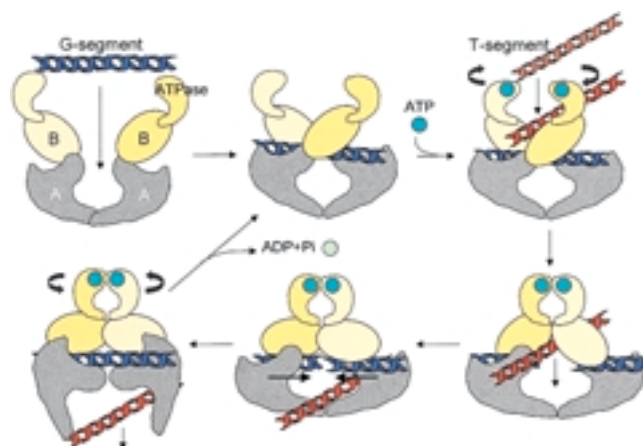

Fig. 2 Mechanism of action of DNA gyrase. DNA gyrase binds a small (approx. 120 bp) DNA segment (G-segment), presumably by wrapping it around the holoenzyme. After binding of ATP the B-subunits dimerize thereby trapping a distant DNA segment (T-segment) within the enzyme. A 4 bp staggered double-strand break is introduced into the G-segment. Transiently, the resulting free 5′-phosphate ends of both DNA strands are covalently attached to conserved tyrosine residues (Y-122) of the A subunits via phosphate ester bonds. This results in the formation of an enzyme-operated DNA gate. The T-segment is passed through this DNA gate and then released. The DNA break is resealed and the initial conformation of the enzyme is restored at the cost of ATP hydrolysis. [Modified according to (7)].

DNA molecule). The ubiquitous distribution among all bacteria and the uniqueness of the supercoiling reaction makes DNA gyrase an "ideal" lethal target for broad-spectrum antibiotics.

Inhibitors of Bacterial Type II Topoisomerases

Several inhibitors of DNA gyrase and/or topo IV have been identified so far. These include (i) the GyrA-targeting 3.2 kD glycine-rich peptide MccB17, which has been isolated from enterobacteria carrying the corresponding biosynthetic gene cluster on a plasmid (10), (ii) the cyclic peptide cyclothialidine isolated from *Streptomyces* and its derivative GR122222X (Fig. 3), which interfere with binding of ATP to the GyrB subunit (11), (iii) cinodine (Fig. 3), a glycocinnamoylspermidine antibiotic, which is produced by *Nocardia* species (12) and targets gyrase as indicated by studies with cinodine-resistant mutants of *E. coli* (13), (iv) clerocidin (Fig. 3), a terpenoid antibiotic from *Fusidium viridae*, which beside its cytotoxic activity on mammalian topoisomerase II also acts on the DNA gyrase A-subunit (14), (15) (Fig. 3).

Since topo IV has only recently been identified as a second quinolone target, most of these drugs have not yet been investigated for their activity on this enzyme. There is one report on the inhibitory activity of the glycosylated flavonoids rutin and isoquercitrin preferentially on topo IV (16).

None of these compounds has yet been developed for clinical use as antimicrobials, probably due to high toxicity (activity on eukaryotic targets), unfavourable pharmacokinetic properties (high molecular weight causing low membrane permeability) or risk of cross-resistance (overlapping binding sites).
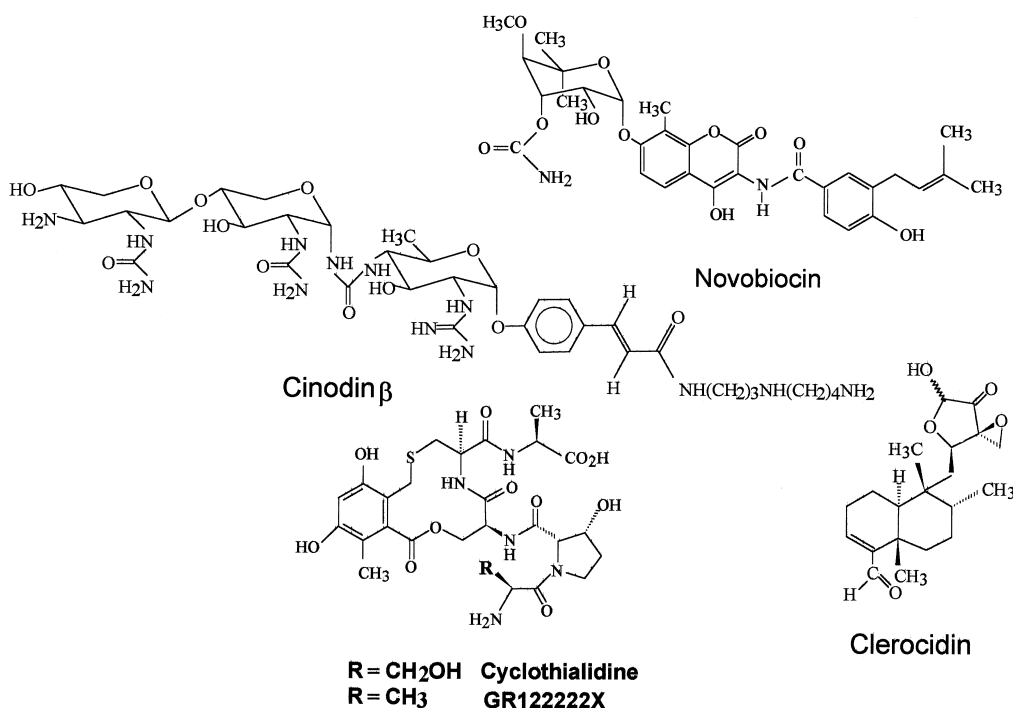
Fig. 3 Inhibitors of DNA gyrase.

In contrast, two classes of inhibitors of bacterial type II topoisomerases, coumarin antibiotics and quinolones, are successfully used for treatment of infections due to Gram-positive and Gram-negative pathogens.

The coumarin antibiotics novobiocin (Fig. **3**), chlorobiocin, and coumermycin A₁, which are naturally produced by *Streptomyces* species, interact with the N-terminal amino acids of the GyrB subunit thereby stabilizing an enzyme conformation of low affinity for ATP (17), (18), (19). Due to their high molec-

ular masses, coumarin antibiotics do not sufficiently penetrate the outer membrane of most Gram-negative bacteria (20). Thus, they are of minor clinical importance and are rarely used for the treatment of infections caused by some Gram-positive pathogens.

Quinolones are synthetic compounds first described in 1962 by Lesher and coworkers. Nalidixic acid (Fig. **4**), the prototype of the quinolone class of antimicrobials, actually is an aza-derivative (1,8-naphthyridine) of 4-quinolone-3-carboxylic acid.
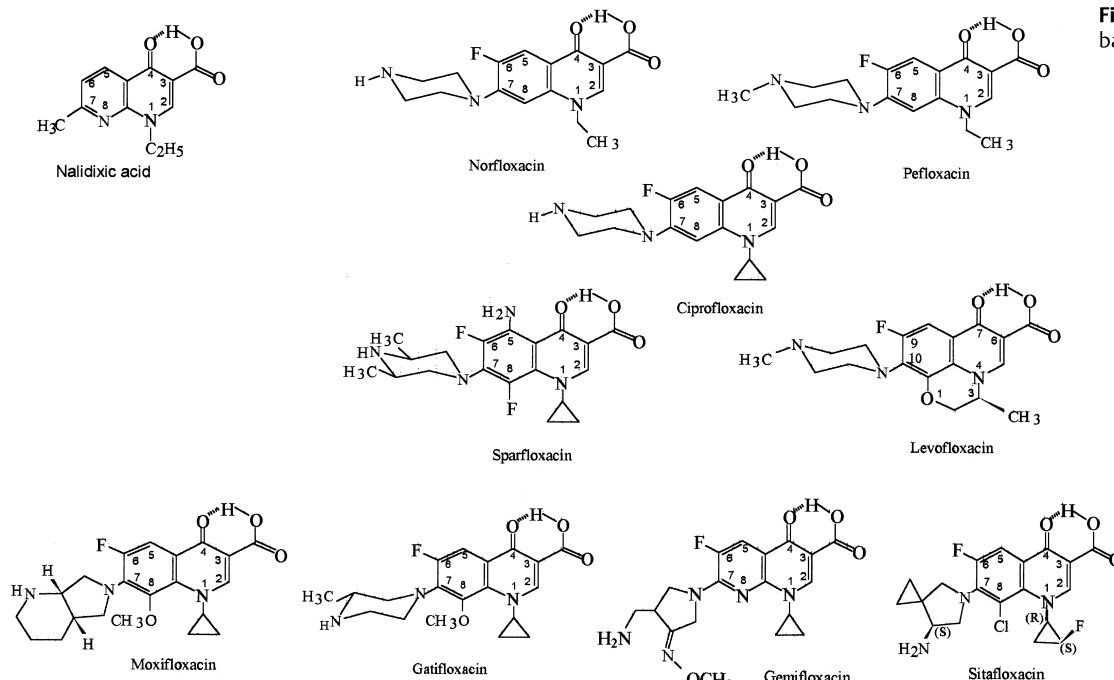

Fig. 4 Quinolone antibacterials.

Table 1 Quinolone susceptibilities of various bacterial pathogens.

Species	Nalidixic acid	Norfloxacin	Ciprofloxacin	Clinafloxacin
		MIC$_{90}$ [μg/ml]		
E. coli	4	0.125	0.015	0.008
P. aeruginosa	>128	4	0.25	0.125
A. baumanii	8	8	0.5	0.125
H. influenzae	1	0.06	0.015	0.004
H. pylori	n.d.*	n.d.	0.5	0.125
M. pneumoniae	n.d.	16	0.5	0.03
B. fragilis	512	128	8	2
S. aureus	64	2	0.	0.06
S. pneumoniae	>128	16	2	0.5
E. faecalis	>128	8	2	1
M. tuberculosis	128	2	1	0.25

* n.d.: not data.

It is derived from a by-product of the chloroquine synthesis (21). Nalidixic acid shows clinically relevant drug concentrations only in urine and has a narrow range of activity against some Gram-negative enterobacteria, restricting its clinical use to the treatment of enterobacterial urinary tract infections. Attempts to broaden its antibacterial spectrum of activity led to norfloxacin, a 4-quinolone-3-carboxylic acid carrying a 6-fluoro and a 7-piperazinyl substituent (Fig. **4**, Table **1**) (22). In addition to an at least 100-fold increase in the antibacterial activity against Gram-negative bacteria, norfloxacin has also a weak, though significant activity against *Staphylococcus aureus* (23). Heteroyclic substituents at the C7-position, e.g., gemifloxacin have improved the activity against Gram-positives (Fig. **4**, Table **1**). another significant improvement came from variations at positions C1 and C8 leading to sitafloxacin, moxifloxacin or gatifloxacin (Fig. **4**).

According to their clinical indication and application, Naber et al. recently suggested to divide fluoroquinolones into four classes (Table **2**) (24). While ciprofloxacin is the most active fluoroquinolone against Gram-negative pathogens including *Pseudomonas aeruginosa*, the new derivatives, e.g., those belonging to classes III and IV are also active against various Gram-positives, like *S. aureus*, *S. pneumoniae* (25), (26), (27), (28), (29), (30), as well as *Mycobacterium tuberculosis* (31), (32), (33) (Table **1**). Thus, the new fluoroquinolones have a high efficacy in the treatment of respiratory tract infections covering not only the common pathogens *S. pneumoniae*, *Hemophilus influenzae*, and *Moraxella catarrhalis*, but also the uncommon *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae* (34).

Mechanism of Action of 4-Quinolones

Quinolone antibacterials display bactericidal activity against dividing cells (Fig. **5a**) resulting in a rapid decrease of the viable cell count by several orders of magnitude (35). This is due to the inhibition of the replicative DNA synthesis rather than protein or RNA synthesis (Fig. **5b**) (36). Evidence for DNA gy-

Table 2 Classification of fluoroquinolones (24).

I	Oral application, exclusively used for urinary tract infections (UTI)	Norfloxacin Pefloxacin
II	Systemic application, wide indication (UTI, respiratory tract infections (RTI), bone and soft tissue infectiosn, sepsis)	(Enoxacin, oral) Fleroxacin Ofloxacin Ciprofloxacin
III	Increased activity against Gram-positive and "atypical" (mycobacteria) RTI, bone infections	Levofloxacin Sparfloxacin (Grepafloxacin)
IV	Like III + anaerobes RTI	Gatifloxacin* Gemifloxacin* (Trovafloxacin) Moxifloxacin (Clinafloxacin)* Sitafloxacin*

* Not yet licensed, (withdrawn).

rase as the target of quinolones came from Gellert et al., who found the inhibitory concentration of oxolinic acid for DNA gyrase isolated from a quinolone-susceptible strain to be much lower than that of its quinolone-resistant derivative carrying a mutation in the structural gene *gyrA*, formerly called *nalA* (Fig. **5c**) (37).

Quinolones act by formation of a stable ternary complex consisting of (i) DNA, covalently attached to (ii) DNA gyrase A subunits of the A$_2$B$_2$ tetramer, and (iii) quinolones thereby preventing the religation step. As a consequence the progress of DNA- and RNA-polymerases along the DNA is blocked. A stop of DNA replication induces the SOS response (38), which triggers the synthesis of new proteins resulting in an arrest of cell division, cessation of the respiratory chain and finally in cell death by an as yet incompletely understood mechanism (39).

Mechanisms of Bacterial Resistance to Quinolones

In general, two basic genetic events can lead to antibiotic resistance – acquisition of additional DNA coding for a resist-

ance determinant and alteration of existing genetic information, e.g., by a mutation. In case of the quinolones, transfer of clinically relevant resistance determinants has not yet been reported.

First experiments to select single-step mutants of *E. coli* with reduced susceptibility to nalidixic acid yielded two types of mutants (40): *NalA*-type mutants show a significant increase of the minimal inhibitory concentration (MIC) of all quinolones, while *nalB*-type mutants show only a moderate increase in the MICs of quinolones, but a reduction in the quino-
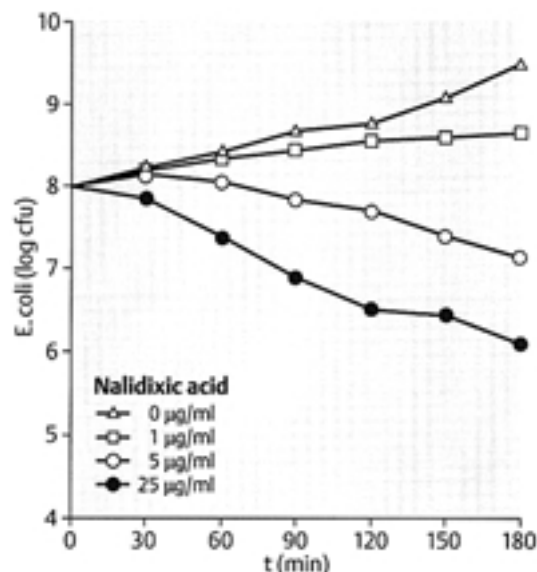

Fig. 5 a Bactericidal activity of quinolones. Addition of nalidixic acid in different concentrations (1, 5, and 25 μg/ml) to exponentially growing cells of a quinolone-susceptible strain of *E. coli* resulted in a concentration-dependent reduction in the viable cell count during 180 min. These data indicate the bactericidal mechanism of quinolones. [Data according to results of (35)].
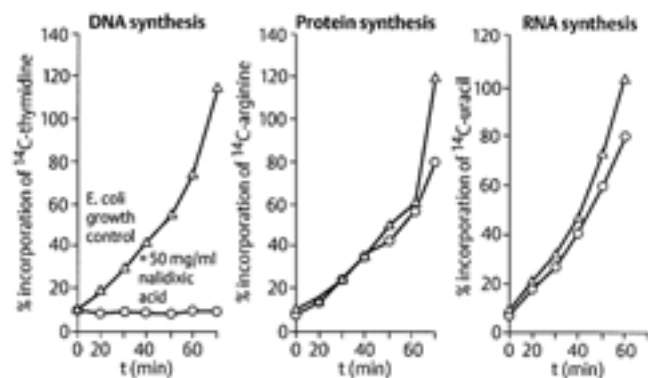

Fig. 5 b Mechanism of action of quinolones – inhibition of DNA synthesis. The addition of nalidixic acid (final concentration 50 μg/ml, ○–○) did not affect the incorporation of radio-labelled precursors of protein synthesis (^{14}C-arginine, center) and of RNA synthesis (^{14}C-uracil, right) by cells of *E. coli* compared to an untreated control (△–△). In contrast, the incorporation of ^{14}C-thymidine (left) is abolished immediately after the addition of nalidixic acid indicating that quinolones are inhibitors of DNA replication. [Data according to results of (36)].
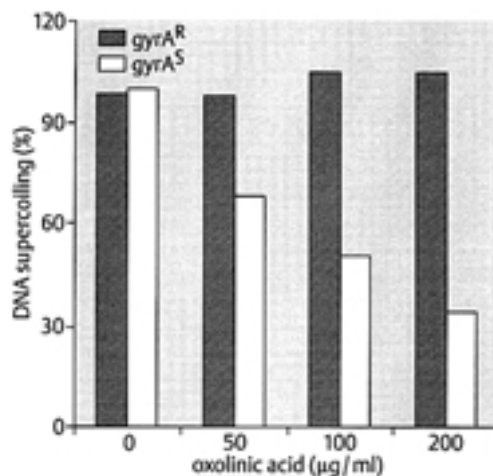

Fig. 5 c Molecular mechanism of action of quinolones – inhibition of DNA gyrase. The enzymatic supercoiling activity of DNA gyrase (*gyrA*[S], white columns) isolated from a quinolone susceptible isolate of *E. coli* is inhibited by the addition of oxolinic acid in a concentration dependent manner. No effect is observed with DNA gyrase (*gyrA*[R], shaded columns) isolated from an *E. coli* strain carrying a single mutation in the gene *gyrA* (formerly *nalA*) coding for a quinolone-resistant A subunit. [Data according to results of (37)].

lone concentration required for inhibiting DNA replication after EDTA treatment, indicating that *nalB* is a transport mutant. No bacterial mechanism capable of inactivating quinolones has been reported so far, although some wood-rotting fungi have been demonstrated to significantly contribute to the degradation of fluoroquinolones in the environment (41).

In contrast to the older, unfluorinated quinolones growth of both types of mutants, *nalA* and *nalB*, can be inhibited by new fluorinated quinolones at clinically relevant concentrations. As a consequence, using even high inocula of about 10^{12} cells it is impossible to select single-step mutants of *E. coli* with clinically relevant resistance to fluoroquinolones (42).

The lack of resistance gene transfer and enzymatic drug inactivation together with the high activity of fluoroquinolones against single-step mutants of naturally quinolone-susceptible bacteria, like *E. coli*, led some investigators to deny the ability of these bacteria to develop clinically relevant resistance towards fluoroquinolones.

Obviously, this optimistic point of view underestimated the high genetic variability of bacteria: During the last decade an increase in the prevalence of fluoroquinolone resistant isolates of *E. coli* from less than 0.5% to about 10% has occurred (43). Such highly resistant clinical isolates show increases in the MICs of, e.g., ciprofloxacin from 0.015 μg/ml for a susceptible isolate to 64 μg/ml and higher for resistant isolates (44), (45), (46), (47).

Target-Mediated Resistance Mechanisms in Gram-Negatives

To elucidate the molecular basis for high-level fluoroquinolone resistance, sequential mutants of a wild-type quinolone-susceptible isolate (WT) have been selected *in vitro*. Three se-

Table 3 Impact of GyrA double mutation S83L+D87G on ciprofloxacin (cip) susceptibility of isolated enzyme$^{(gyrase)}$ and whole cells$^{(wc)}$.

Strain	GyrA-mutation	IC$_{90}$$^{gyrase)}$ cip [μg/ml]	MIC$^{(wc)}$ cip [·g/ml]
WT	–	1	0.015
M I	S83L	10	0.5
M I*	D87G	10	0.25
M III	S83L+D87G	>2000	64 (selected *in vitro*)
WT-3	S83L+D87G	>2000	1 (mutagenized)
321	S83L+D87G	>2000	64 (clinical isolate)

* Obtained by *in-vitro* mutagenesis.

lection steps yielding mutants MI, MII, and MIII were necessary to obtain high-level resistance (MIII with MIC of ciprofloxacin of 64 μg/ml) (48). Results of a genetic dominance test (49), which is based upon the dominance of a – plasmid-coded – quinolone susceptible over the – chromosomally encoded – resistant allele of *gyrA* (49) or *gyrB* (50) indicated the involvement of *gyrA*, but not *gyrB* mutations (48), (51). In addition, mutant MII carries an *nalB*-like mutation displaying a *m*ultiple *a*ntibiotic *r*esistance (*mar*) phenotype (52) that affects susceptibility not only to quinolones – as indicated by a reduction in the accumulation of ciprofloxacin by about 70% – but also to unrelated drugs, like chloramphenicol (53).

Determining for WT and its derivatives the DNA sequence of the so-called "quinolone resistance-determining region" (QRDR) of *gyrA*, where all known single step mutations associated with quinolone resistance map (54), revealed a serine-83 to leucine (S83L) mutation in mutant MI and, in addition, an aspartate-87 to glycine (D87G) mutation in mutant MIII (55). DNA supercoiling activity of DNA gyrase isolated from mutant MIII is as refractory to ciprofloxacin (IC$_{90}$ > 1,500 μg/ml) as that of DNA gyrase reconstituted from a wild type GyrB subunit and a genetically engineered GyrA subunit containing solely the double mutation S83L+D87G (Table **3**). Expanding the DNA sequence analyses to clinical isolates with high-level fluoroquinolone resistance revealed that it is associated with a *gyrA* double mutation primarily affecting codons for serine-83 and aspartate-87 (53). In order to investigate if this double mutation is sufficient for the expression of high-level fluoroquinolone resistance, the susceptibilities to ciprofloxacin were determined for mutant MIII selected *in vitro* and mutant WT-3 which was obtained by *in vitro* mutagenesis. WT-3 contains only the *gyrA* double mutation S83L+D87G in the genetic background of WT. Mutant WT-3 was as susceptible as the single step mutant MI (Table **3**), indicating the presence of an additional, yet unidentified mutation in MIII. Data of Hoshino et al. (56) demonstrating that fluoroquinolones inhibit *in vitro* the activity of topo IV at concentrations several fold higher than those required for inhibition of DNA gyrase, supported the idea that topo IV is an additional target of fluoroquinolones in *E. coli*.

This hypothesis was challenged by determining the DNA sequence of a fragment from *parC* containing the region homologous to the QRDR of *gyrA* for the susceptible parent strain WT and its derivatives MI, MII, and MIII. Compared to WT no alteration was found for MI and MII, however, mutant MIII did carry a mutation resulting in a serine-80 to isoleucine change. Serine-80 of ParC is homologous to serine-83 for GyrA (Fig. **6**).

Fig. 6 Conserved amino acid sequences in GyrA and ParC of various Gram-negative bacteria and quinolone resistance mutations. According to the respective proteins from *E. coli* amino acids of the quinolone resistance-determining region [QRDR, (54)] of subunit A of DNA gyrase (GyrA, top) and the homologous region of subunit A of topoisomerase IV (ParC, bottom) were numbered. Naturally variant amino acids of the respective genes detected in quinolone-susceptible strains of several Gram-negative species are given below the respective QRDR. Mutations associated with quinolone resistance in various Gram-negative isolates in subunits A of both target topoisomerases are shown under below the line the respective amino acid positions (44), (45), (46), (47), (53), (60), (92), (93), (94).

Subsequent DNA sequencing of clinical isolates with high-level fluoroquinolone resistance revealed the presence of at least one mutation within the QRDR-like region of *parC*. All amino acids in ParC associated with quinolone resistance were homologous to those in GyrA (Fig. **6**). Moreover, transfering a plasmid-coded quinolone-susceptible allele of *parC* obtained from strain WT into *parC* mutant strains (e.g., MIII) resulted in a reduction of the fluoroquinolone resistance to the level of a single *gyrA* mutant, but not to that of WT. This dominance effect was not observed if the *parC* gene obtained from mutant MIII was transferred (44).

These results indicating the requirement of a combiantion of mutations affecting both targets of quinolones, topo IV and DNA gyrase, are required for high-level resistance, do not only apply to *E. coli* (57), (58), (59), but also to other Gram-negative pathogens (60). Strains carrying a combination of three target mutations are resistant even to new derivatives like clinafloxacin, sitafloxacin showing highest antibacterial activity (61).

Target-Mediated Resistance Mechanisms in Gram-Positives

Ciprofloxacin and ofloxacin carrying modified N1-substituents were the first fluoroquinolones to be licensed for a broad indication including infections of the bone, the skin, and the soft tissue, which can be caused by *S. aureus*, a Gram-positive pathogen. However, the antibacterial activities of these fluoroquinolones against Gram-positives are weaker than against most Gram-negatives (Table **1**). Thus, clinically resistant mutants of *S. aureus* can be obtained not only in a single step *in vitro* but also frequently from patients receiving fluoroquinolone therapy. Molecular genetic analysis of the underlying mechanism of resistance in *S. aureus* revealed that topo IV is the primary, more sensitive target, while DNA gyrase is secondary (62). One approach to improve the activity against Gram-positives was the chemical modification of the C8 position: Introducing a -F, a -Cl, or a -OCH$_3$ substituent yielded

fluoroquinolones, like sparfloxacin, sitafloxacin, and moxifloxacin (Fig. **4**) with enhanced activities against Gram-positives and several mycobacterial species, while retaining most of the activities against Gram-negatives (Table **1**). However, a halogen substitutent is associated with phototoxicity limiting the use of, e.g., sparfloxacin and clinafloxacin (63). In contrast, no such side effects have been reported for moxifloxacin with a -OCH$_3$ substitution.

In vitro data indicate that topo IV is the primary target of fluoroquinolones in *Enterococcus faecalis* (64), (65) and *S. pneumoniae*, too (66), although for *S. pneumoniae* the target preference seems to depend on the structure of the fluoroquinolone (67).

Resistance due to Altered Drug Transport

Accumulation of fluoroquinolones in the cytoplasm at relevant concentrations is due to both the chelating activity of the 4-oxo-3-carboxylic acid moiety, which destabilizes the lipopolysaccharide layer of the outer membrane (68), and the zwitterionic character of these drugs, allowing them to pass the cytoplasmic membrane by exploiting the proton gradient (Fig. **4**) (69).

Drug accumulation in a Gram-negative bacterial cell can be reduced by two different mechanisms: (i) reduction of the drug influx into the cell through the outer membrane, the major barrier for hydrophilic molecules, and (ii) increase in drug efflux out of the cell by energy-driven transmembrane export (Fig. **7**) (70).
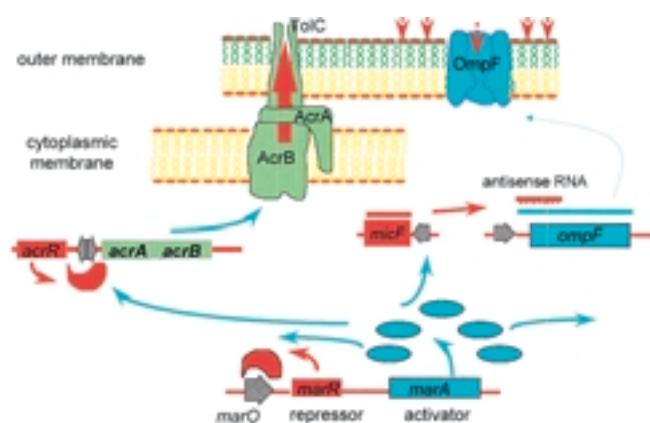

Fig. 7 Multiple antibiotic resistance in *E. coli*. The *mar* operon contains a transcriptional unit of three genes *marR*, *marA*, and *marB*, which are under negative control of the MarR repressor (95). Environmental stimuli, like salicylic acid or bile salts as well as mutations inactivating repressor activity (86) result in constitutive overexpression of MarA. This, in turn, activates several unrelated genes including that encoding the *micF* antisense RNA complementary to the 5′-end of the *ompF* mRNA and those encoding the three-component efflux pump AcrAB-TolC capable of extruding antibiotics like quinolones or chloramphenicol (96). Thus, the intracellular concentration of quinolones is significantly reduced by simultaneously decreasing drug influx (via OmpF) and increasing drug efflux (via AcrAB-TolC) to the extracellular space. AcrB is the pump spanning the cytoplasmic membrane, TolC is an unspecific outer membrane protein, and AcrA is the membrane fusion protein, which spans the periplasmic space and thereby connects the pores formed by AcrB and TolC. A local repressor AcrR controls the expression of the acrAB genes in the absence of MarA (84), (97).

Some less hydrophilic quinolones like nalidixic acid are assumed to partially diffuse directly through the lipid bilayer of the outer membrane (71). However, diffusion of small (M_r < 700) hydrophilic molecules, usually nutrients, occurs through porins, channel-forming proteins in the outer membrane. One of the best studied porins is the trimeric OmpF porin of *E. coli*, which is the major entry site for fluoroquinolones (72). Beside various environmental factors, e.g., osmolarity, temperature, pH, which can modulate the expression of OmpF (73), mutations abolishing the function and/or the expression of OmpF have been shown to result in reduced susceptibility to fluoroquinolones and unrelated drugs (74), (75). Compared to a *gyrA* mutation the loss of OmpF porin function has less impact on the quinolone resistance (76).

Increased drug export has been identified as a widely distributed mechanism of broad-spectrum resistance to antibiotics. One of the best-studied systems of antibiotic export is that controlled by the *mar*-operon of *E. coli* (77) which negatively controls the expression of OmpF and positively controls the expression of the efflux pump AcrAB-TolC (Fig. **7**). This pump belongs to the RND efflux systems, one of four known major classes of transport systems. Several homologous efflux pumps involved in quinolone resistance have been identified in Gram-negative enterobacteria (78), *Neisseria gonorrhoeae* (79), and *P. aeruginosa* (80), (81), (82), (83). While efflux pumps significantly contribute to the natural multiple-drug resistance of and have a high impact on fluoroquinolone resistance in *P. aeruginosa*, their role for quinolone resistance in other Gram-negatives is less well established (84). Increased efflux is found in clinical isolates as well as in laboratory mutants, but is associated with a much lesser increase in resistance compared to target-mediated mutations (85). The genetic basis for increased efflux is poorly understood and in *E. coli* mutations affecting the global regulatory component MarR or local repressors, like AcrR can be involved (Fig. **7**) (86).

In Gram-positives drug efflux pumps belonging to the major facilitator family (87) have also been identified as factors modulating the fluoroquinolone resistance. These include NorA of *S. aureus* (88), (89) and PmrA of *S. pneumoniae* (90), which can be inhibited by reserpine and predominantly affect the activities of hydrophilic compounds, like norfloxacin and ciprofloxacin, but not those of more hydrophobic derivatives, like sparfloxacin or moxifloxacin (91).

Outlook

Within 15 years the fluoroquinolone class of antibacterial agents has become one of the most important drug classes showing high bactericidal activity against a broad range of Gram-positive and Gram-negative pathogens. Combined with favorable pharmacokinetics the pharmacodynamic features of the fluoroquinolones made these drugs most powerful chemotherapeutic agents for nearly all kinds of infections. However, clinically relevant resistance has developed even in strains belonging to highly susceptible species, most probably due to a high selective pressure by inappropriate use of these drugs. Thus, strictly regulated indications for use are necessary to avoid rapid development of resistance and to conserve this potential for the future. While the synthetic quinolone antimicrobial drugs are advantageous over naturally occurring by lack of both enzymatic inactivation mechanism and

transferable resistance it would be naive thinking that development of resistance is impossible. A promising way of saving the antimicrobial potential of new drugs is their prudent use for strictly defined indications and a continuing monitoring of resistance development.

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