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Quinolone Resistance in Tuberculosis Treatment: A Structural Overview

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1. Introduction

1.1 Fluoroquinolones in tuberculosis treatment

Fluoroquinolones are key antibiotics for the second-line treatment of multidrug-resistant tuberculosis (MDR-TB), i.e. resistant to the two most powerful antituberculous drugs currently available, isoniazid and rifampicin (WHO, 2001; 2011). Unfortunately, extensively drug resistant strains (XDR-TB), defined as MDR-TB with bacillary resistant to any fluoroquinolones and to one of the three injectable second line anti-TB drugs (amikacin, kanamycin or capreomycin), are emerging worldwide (Center for Disease Control and Prevention, 2006). XDR-TB strains constitute a major concern for public health since they are virtually untreatable and responsible for up to 100% of deaths. Besides being increasingly popular in treatment of tuberculosis complicated by intolerance or by relative contraindication for first-line drugs, fluoroquinolones are under studies to shorten the duration of treatment or replace first line drugs in susceptible tuberculosis. Newer fluoroquinolones such as moxifloxacin have already demonstrated potential for shortening treatment duration (Rustomjee et al., 2008; Conde et al., 2009; Dorman et al., 2009).

Fluoroquinolones are also one of the most widely prescribed antibiotics as they are generally well tolerated with high oral bioavailability plus broad-spectrum antibacterial activities against genitourinary infections and against common respiratory tract pathogens. Structurally, these drugs contain a quinoline ring system and hence are given the name quinolones (Fig. 1). After the first generation of drugs were found to be active, it was noted that a fluorine atom at the 6-position of the quinoline ring imparted greater potency, and hence the second generation of drugs was called the fluoroquinolones (Fig. 1).

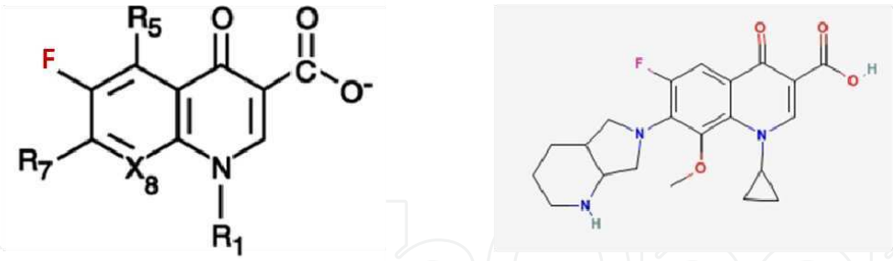

Fig. 1. Chemical structures of a fluoroquinolone (left) and moxifloxacin (right). The fluorine atom, signature of fluoroquinolones, is at position 6 in the quinoline ring. In the moxifloxacin structure, oxygen atoms are coloured in red, nitrogen atoms in blue, hydrogen atoms in green and the fluor atom in purple. R1 is a cyclopropyl group, R5 is a hydrogen atom, R7 is an azabicyclo group and X8 a methyl ether group.

2. Bacterial type II topoisomerases are the target of fluoroquinolones

Topoisomerases are ubiquitous nucleic acid-dependant nanomachines essential to cell life and solve the topological problems of DNA that occur as a result of DNA manipulations during replication, transcription, recombination, chromosome segregation and condensation, maintenance of nuclear architecture and apoptosis (Champoux, 2001). They have been divided into two classes, type I and type II, according to their basic mechanism of action (Champoux, 2001; Forterre et al., 2007). Type I DNA topoisomerases introduce transient single-stranded breaks to force the passage of one DNA strand through the other, whereas type II DNA topoisomerases introduce transient double-stranded breaks to force the passage of a second DNA duplex through this broken duplex. All organisms contain at least one type I and one type II topoisomerases. Type I and type II topoisomerases can sometimes perform similar function *in vivo*, for example efficient separation of interlocked chromosomes at the end of DNA replication via their decatenation activity (Nurse et al., 2003). All type II topoisomerases can also relax both positive and negative superturns that accumulate during transcription and replication. Topoisomerases thus play an essential role for the preservation of genome stability in all life forms (Forterre et al., 2007).

Type II topoisomerases have been further sub-classified into two families, type IIA and IIB, based on evolutionary considerations (Champoux, 2001; Forterre et al., 2007). The type IIA includes bacterial DNA gyrase and topoisomerase IV, eukaryal and viral topoisomerases, whereas the type IIB includes archaeal topoisomerase VI, and their homologues in plants, a few protists, and a few bacteria (Forterre et al., 2007; Forterre & Gadelle, 2009). Type IIA and IIB topoisomerases are both formed by the association of two subunits, sharing homologous sequences and domain organization (Fig. 2). Bacterial type II topoisomerases, DNA gyrase and topoisomerase IV, consist of two subunits, GyrA or ParC and GyrB or ParE, which form the catalytic active heterotetrameric (A_2B_2/C_2E_2) complex. Subunit A consists of two domains, the N-terminal breakage-reunion domain and a carboxy-terminal domain, termed BRD and CTD, respectively (represented in blue and green respectively in Fig. 2 and 3). Subunit B consists of the ATPase domain followed by the Toprim domain (represented in yellow and red, respectively, in Fig. 2 and Fig. 3). The GyrB Toprim domain and GyrA BRD come from separate subunits and cooperatively form the enzyme catalytic core (see section

5). The BRD contains the catalytic tyrosine responsible for the cleavage and religation of the DNA double helix.

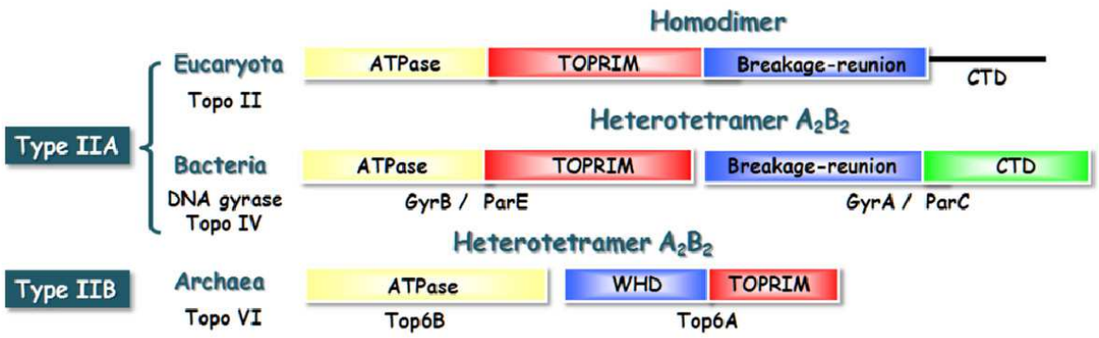

Fig. 2. Schematic representation of the sequence and domain organization of type II topoisomerases found in eukaryotes, bacteria, virus and archaea. The type IIA topoisomerase family includes bacterial DNA gyrase and topoisomerase IV (Topo IV), eucaryal and viral topoisomerases (Topo II), whereas the type IIB topoisomerase family includes archaeal topoisomerase VI (Topo VI), and their homologues in plants and a few protists and bacteria. Topo IIA and Topo IIB are both formed by the association of two subunits A and B. Bacterial type IIA and type IIB topoisomerases are A_2B_2 heterotetramers, whereas the eukaryotic Topo II is a heterodimer, with the A and B subunits fused in a single polypeptide (Nter-B-A-Cter). The names of the four conserved domains are indicated. The winged helix domain (WHD) in the type IIB topoisomerase corresponds to the breakage-reunion domain (BRD) of type IIA topoisomerase.

Crystallographic studies of individual domains show that the structure of the BRD is a heart-shaped arrangement with two dimer interfaces (Morais-Cabral et al., 1997), that the CTD displays a spiral six-bladed β-pinwheel structure (Corbett et al., 2004) and that the ATPase domain belongs to the GHKL ATPases, a broad family of enzymes with a common fold unrelated to other canonical ATP-binding folds (Brino et al., 2000) (Fig. 3). The Toprim domain interacts with the A subunit, possesses a magnesium-binding site and is essential for DNA binding (Fig. 3). Although no structure for a fully intact active type IIA topoisomerase has been determined yet, combination of structural and biochemical studies of the individual domains has lead several authors to propose a global quaternary structure model and a catalytic mechanism of the holoenzyme (Schoeffler & Berger, 2008). The BRD binds a DNA segment termed the 'gate-' or G-segment at the DNA-gate (Fig. 3). The N-terminal ATPase domains dimerize upon ATP binding, capturing the DNA duplex to be transported (T-segment). The T-segment is then passed through a transient break in the G-segment opened by the breakage-reunion domains, the DNA is resealed and the T-segment released through a protein gate, the C-gate, prior to resetting of the enzyme to the open clamp form (Fig. 3).

Quinolones, which target the two bacterial type II topoisomerases, exert their powerful antibacterial activity by interfering with the enzymatic reaction cycle. Specifically, they bind to the enzyme-DNA binary complex, thereby stabilizing the covalent enzyme tyrosyl-DNA phosphate ester (see also section 6 and Fig. 7). The resulting ternary complexes block DNA

replication and lead to cell death (Hooper, 2003). In addition, hydrolysis of this linkage leads to the accumulation of double-stranded DNA fragments, which accounts for the bactericidal activity.

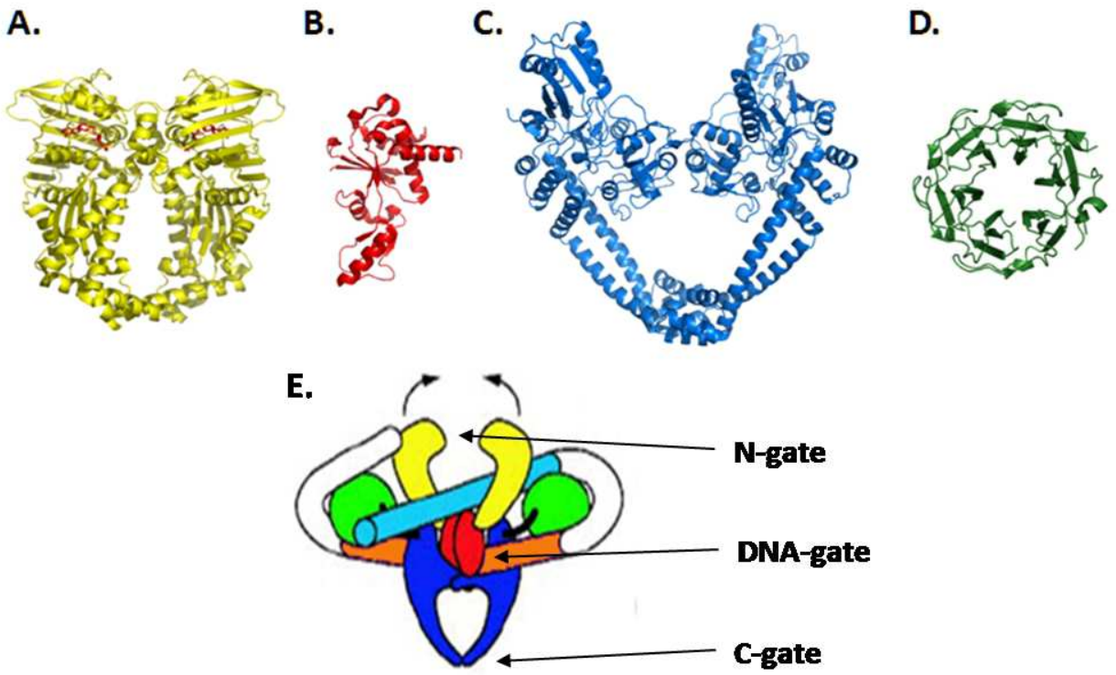

Fig. 3. Crystal structures of the individual domains of type II topoisomerases, as defined in Fig. 1. Structures of the GHKL domain (A), Toprim (B), BRD (C) and DNA gyrase CTD (D) domains. Two ATP molecules are represented in red in (A). (E) Global architecture of type IIA topoisomerases proposed based on the structures of the isolated domains adapted from Corbett & Berger (2004). Color code is the same than in Fig. 1. The dimerization interfaces of the heterotretramer constitute the three gates (N-, DNA- and C-gate), through which the DNA double helix (T-segment, represented in cyan) will be transported. The G-segment that binds the DNA-gate is represented in orange.

3. *M. tuberculosis* DNA gyrase as the sole target of fluoroquinolones

Bacterial genomes usually encode two type IIA enzymes, DNA gyrase and topoisomerase IV, that together help manage chromosome integrity and topology (Champoux, 2001). DNA gyrase is unique in introducing negative supercoils into DNA, an activity mediated by the CTD of its DNA binding subunit (GyrA) and is therefore responsible for the DNA unwinding at replication forks. Although closely related to DNA gyrase, topoisomerase IV has a specialized function in mediating the decatenation of interlocked daughter chromosomes (Levine et al., 1998) and relaxes positive supercoils. Particularly intriguing is that some bacteria possess in their genome only one type II topoisomerase, DNA gyrase.

Given the important role of DNA supercoiling in DNA replication, transcription, and chromosome dynamics, it is not surprising that DNA gyrase genes have been found in all bacterial genomes sequenced to date. In contrast, the topoisomerase IV gene is absent in a

few bacteria such as *Treponema pallidum*, *Helicobacter pylori*, and notably *Mycobacterium tuberculosis*, the intracellular pathogen that causes tuberculosis (Cole et al., 1998) (Fig. 4). Consequently, these bacteria are unusual in producing a "single" type II topoisomerase. DNA gyrase is therefore the unique target of fluoroquinolones in these organisms, as it has been demonstrated for *M. tuberculosis* (Mdluli & Ma, 2007).

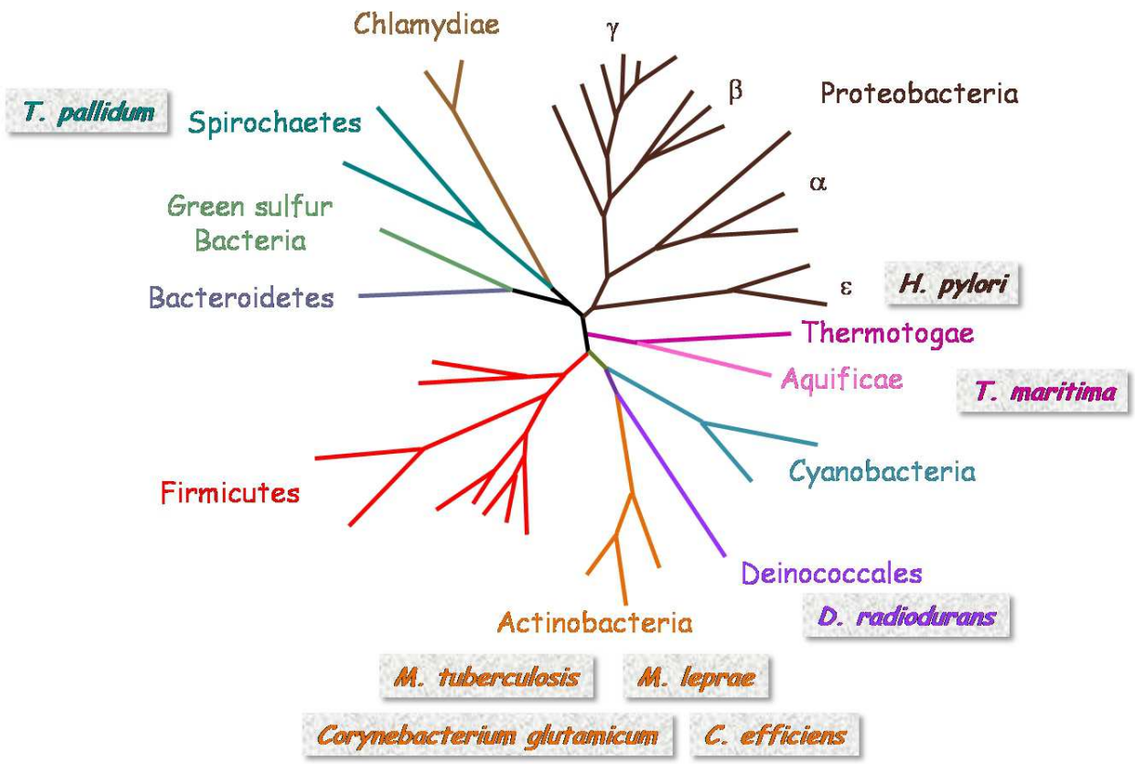

Fig. 4. Bacterial phylogenetic tree showing the distribution of species known to possess only one type II topoisomerase gene in their genome.

The direct consequence of the presence of a unique type II topoisomerase in *M. tuberculosis* has been investigated and it has been shown that *M. tuberculosis* DNA gyrase presents two specificities. First, this enzyme possesses an "hybrid activity". In addition to its normal supercoiling activity (e.g. comparable to that of *E. coli* DNA gyrase), it possesses a decatenation activity superior to that of DNA gyrase from species harboring two type II topoisomerases (Aubry et al., 2006). In addition, it shows an enhanced DNA relaxation and cleavage activities. However, the unique type II topoisomerase of *M. tuberculosis* decatenates kDNA less efficiently than a genuine topoisomerase IV, e.g., that of *S. pneumoniae*. The second specificity concerns the susceptibility to fluoroquinolones. The *M. tuberculosis* DNA gyrase is indeed naturally less susceptible to fluoroquinolones than other bacterial DNA gyrases. We have shown that three residues of the *M. tuberculosis* sequence play an important role in this natural resistance mechanism (Matrat et al., 2006). Replacing M74 in GyrA, A83 in GyrA, and R447 in GyrB of *M. tuberculosis* gyrase by their *E. coli* homologs (Ile, Ser and Lys, respectively, see residues pink underlined in Fig. 5) resulted in active enzymes as quinolone susceptible as the *E. coli* DNA gyrase. However, the question whether the

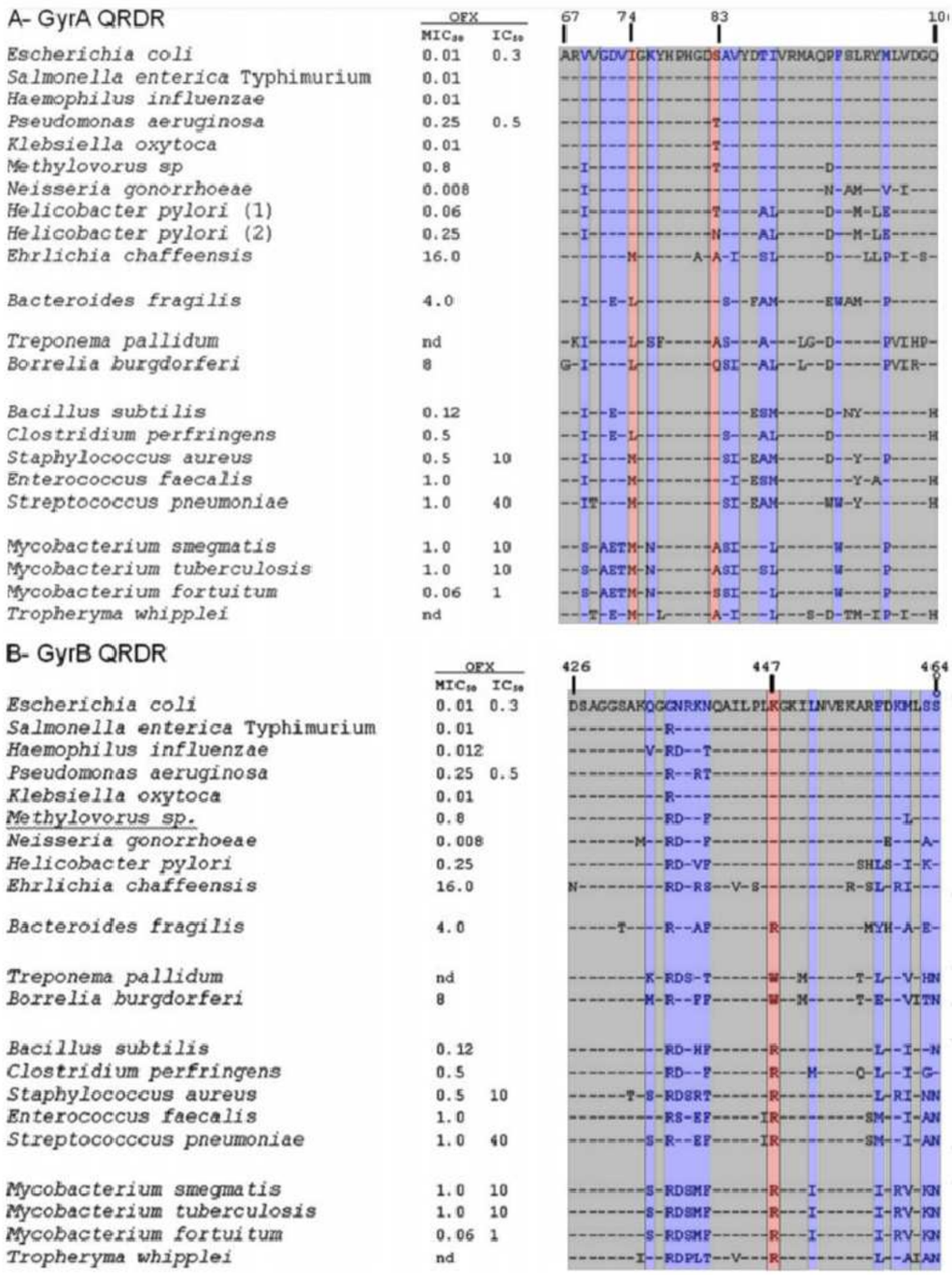

Fig. 5. Two parts of the multiple alignment of subunit A and B, respectively, centred on the quinolone resistance determining regions (QRDR, see section 4 for definition), after Matrat et al. (2008). Residues highlighted in blue underline the most important residues shown to be involved in acquired resistance to fluoroquinolones. Residues highlighted in pink underline residues of the *M. tuberculosis* DNA gyrase sequence involved in the natural resistance mechanism. The numbering at the top of the alignments corresponds to the *E. coli* DNA gyrase numbering. The values of MIC_{50} and IC_{50} are given for ofloxacin when known.

"single" DNA gyrases of *Treponema pallidum* or *Helicobacter pylori* display similar specificities, like increased decatenation activity or lower susceptibility to quinolones, is still open.

4. The quinolone resistance determining regions and the quinolone binding pocket

Bacterial resistance to antibiotics typically involves drug inactivation or modification, target alteration, or decrease in drug accumulation associated with decrease in permeability and increase in efflux (Li & Nikaido, 2004). Fluoroquinolone resistance mechanisms can involve two of these three mechanisms, target alterations and overexpression of intrinsic multidrug resistance (MDR) efflux pumps (Hooper, 2002). However, resistance to fluoroquinolone is mainly due to target modification, e.g. mutations in the DNA gyrase and/or topoisomerase IV genes. Mutations conferring bacterial resistance to quinolones occur in two short discrete segments termed the quinolone resistance-determining regions (QRDR) in the breakage-reunion domain of subunit A (QRDR-A) and more rarely in the Toprim domain of subunit B (QRDR-B) (Hooper, 1999) (Fig. 6).

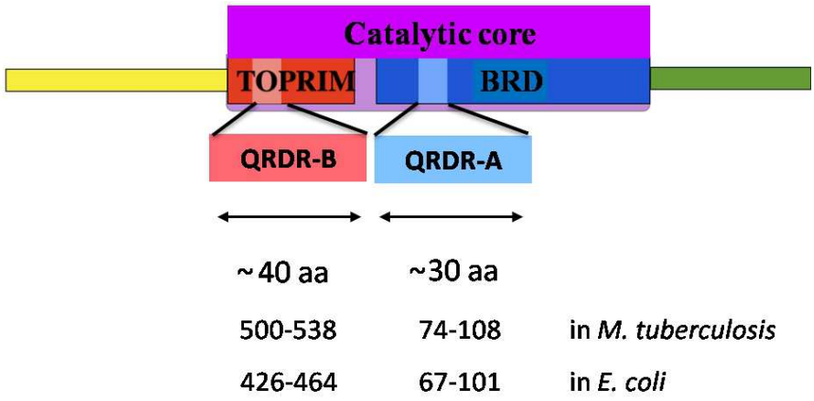

Fig. 6. Schematic representation of the *M. tuberculosis* DNA gyrase sequence. Subunit A contains the breakage-reunion domain (BRD, blue) and the C-terminal domain (CTD, green). The subunit B contains the ATPase domain (yellow) and the Toprim domain (red). Localization of the QRDR is indicated in pink and light blue. The color code is the same than in Fig. 2 and 3. The approximate lengths of the QRDRs are indicated (30 and 40 aa for the QRDR-A and -B, respectively). The residue ranges of the QRDR-A and -B are indicated for the *M. tuberculosis* and the *E. coli* DNA gyrase sequences.

Whereas residues of the QRDR-A have been known for a while that they are spatially close to the catalytic tyrosine responsible for the double-stranded DNA cleavage, residues of the QRDR-B were only hypothetically thought to be involved in the quinolone-binding pocket (Heddle & Maxwell, 2002). This ambiguity was brought about by the two first structures of the catalytic core (an homodimer formed by two single polypeptides composed of the Toprim domain and the BRD) of the yeast topoisomerase II (Fass et al., 1999; Berger & Gamblin, 1996). In these structures, which correspond to two different conformational arrangements of the catalytic core, the two QRDRs are too far from each other to form a unique binding pocket for the quinolone molecule (Fig. 7).

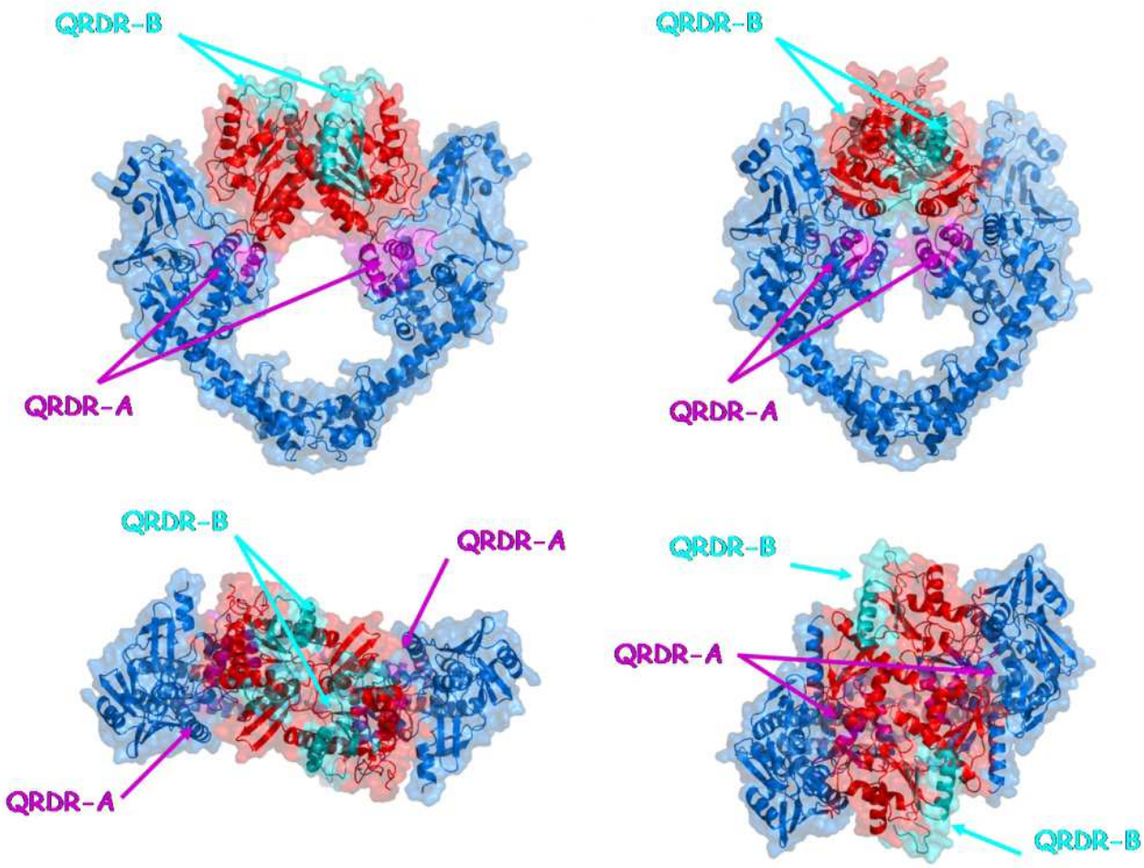

Fig. 7. Side view (top left and right) and top view (bottom left and right) of the catalytic core of the yeast topoisomerase II, composed of the Toprim domain (represented in red) and the breakage-reunion domain (BRD, represented in blue). (Left) "Open" conformation of the catalytic core (PDB code 1BGW, Berger et al., 1996). The DNA-gate is open and the C-gate is closed (for definition of DNA- and C-gate, see Fig. 3). The QRDR-B (represented in cyan) are close together but distant from the QRDR-A (represented in purple). The QRDR-A includes the two DNA-binding helices, H3 and H4. (Right) "Intermediate" conformation of the catalytic core (PDB code 1BJT, Fass et al., 1999). The DNA-gate is slightly open and the C-gate is closed. The QRDR-B are more distant from each other and are closer to the QRDR-A, but still do not form a unique quinolone-binding pocket.

Involvement of QRDR-B residues in the quinolone-binding pocket was definitely confirmed in 2009 with the first structure of the *S. pneumoniae* topoisomerase IV Toprim and breakage-reunion domains in complex with DNA and moxifloxacin (Lapogonov et al., 2009). This structure highlighted the fact that the GyrA BRD and the GyrB-Toprim domain form the catalytic core complex, where the double-stranded DNA interacts at the interface of the two domains (Fig. 8). This structure brought for the first time the structural evidence of the covalent link formed between the DNA and the enzyme, a phosphodiester bond between

the oxygen of the tyrosine and the phosphor atom of a phosphate group (Fig. 8). In addition, this structure also confirmed that the QRDR-A corresponds to the DNA-binding domain of the subunit A often referred to as a CAP-like domain constituted by a helix-turn-helix motif (H3 and H4). The QRDR-B corresponds to the DNA-binding region of subunit B that involves a helix and a long loop (see section 5). However, the main conclusion of this work was that the conformational arrangement observed in this structure brings together the two QRDRs to form a unique quinolone-binding pocket (see section 6) at the interface formed by the DNA, the BRD and the Toprim domain (Fig. 8).

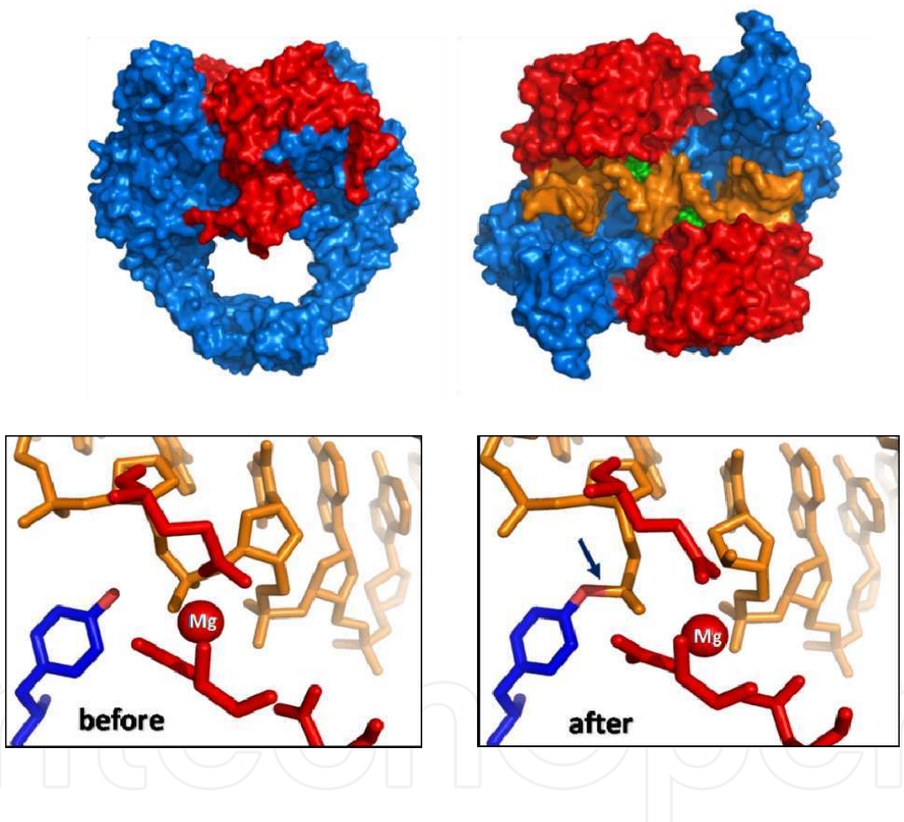

Fig. 8. Crystal structure of the *S. pneumoniae* topoisomerase IV catalytic core that comprises the GyrB Toprim domain and GyrA BRD in complex with DNA and moxifloxacin. The Toprim domain is represented in red, the BRD in blue, the 35 base pairs DNA oligonucleotide in orange and the moxifloxacin in green. (Top) Side and top views of the molecular surface of the catalytic core. (Bottom) Close view of the catalytic tyrosine (represented in blue sticks) before and after formation of the covalent link between the enzyme and DNA (the phosphodiester bond is indicated by a black arrow). Residues of the Toprim domain chelating the magnesium ion are represented in red sticks.

5. Structural studies of the *M. tuberculosis* DNA gyrase catalytic core

We recently solved the crystal structures of two domains of the *M. tuberculosis* DNA gyrase, the GyrB Toprim (TopBK) and the GyrA breakage-reunion (GA57BK) domains (Piton et al., 2009; 2010). These two domains form the DNA gyrase catalytic core that corresponds to the smallest entity able to catalyse DNA cleavage (Piton et al., 2010).

We solved two high resolution structures of the Toprim domain to 2.1 and 1.95 Å resolution, respectively. These two structures display two different conformations of the magnesium binding site. The QRDR-B is constituted by residues 500 to 538 (461-499 in the PDB numbering). Part of this region is disordered and corresponds to a loop that has been named DNA-binding loop (DBL) because it is folded in the presence of DNA (Fig. 9). We obtained four crystal forms of the breakage-reunion domain, diffracting from 4.2 to 2.7 Å resolution depending on the crystal form (Piton et al., 2009). This was the first structure determination report of the BRD of a DNA gyrase from a species containing one unique type II topoisomerase. The structure of this domain is unexpectedly very similar to other structures of BRD solved for bacterial type II topoisomerases, DNA gyrase and topoisomerase IV (Morais-Cabral et al., 1997; Carr et al., 2007). It displays the typical heart-like shaped structure with a hole of 30 Å diameter at the center allowing the passage of the DNA from the DNA-gate to the C-gate (Fig. 9). The QRDR-A is localised at the DNA-gate (shown in purple in Fig. 9).

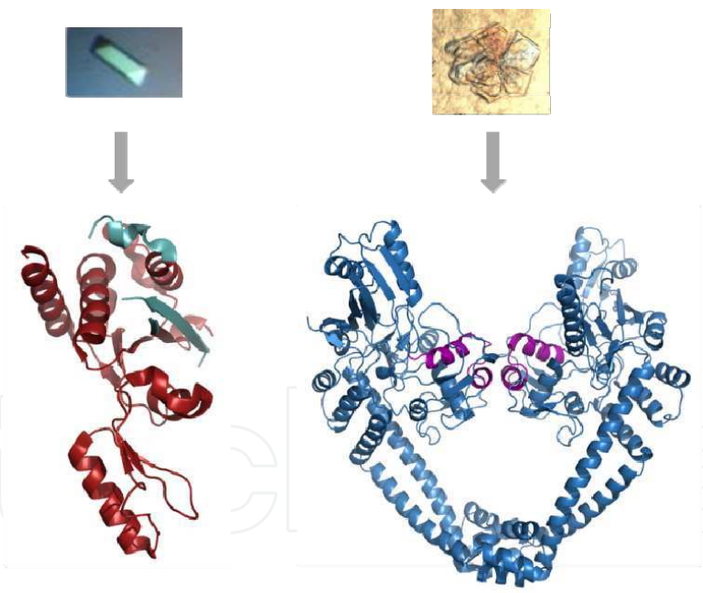

Fig. 9. Crystals (top) and crystal structures (down) of the GyrB Toprim (TopBK, PDB code 3IG0, 242 residues) domain represented in red (left) and the GyrA breakage-reunion (BRD, GA57BK, PDB code 3IFZ, 508 residues per monomer) represented in blue (right). The QRDR-B is represented in cyan in the TopBK structure (residues 500-538), the QRDR-A is represented in purple in the GA57BK structure (residues 74-101).

Using the two crystal structures we determined, the GyrA BRD (GA57BK) and the GyrB Toprim (TopBK) domains, we performed the modeling of the *M. tuberculosis* catalytic core in complex with a 35 base pairs DNA oligonucleotide and the most used fluoroquinolone,

moxifloxacin. In both the crystal structure of the *S. pneumoniae* topoisomerase IV and the modeled *M. tuberculosis* DNA gyrase catalytic cores, the fluoroquinolone molecules are bound at the ternary interface formed by the DNA, the BRD and the Toprim domain (Fig. 8 and 10). The general mode of action known for quinolones, common to DNA gyrase and topoisomerase IV, was the inhibition of the broken DNA religation. The way how these molecules are responsible for this inhibition has been clarified by the structure. Whereas quinolones are not DNA intercalators, the fluoroquinolone molecule is intercalated in the ternary complex between the dinucleotide step where the covalent bond between the DNA and the enzyme (with the catalytic tyrosine) is formed (Fig. 8 and 10). As the catalytic core is dimeric, two fluoroquinolones molecules are bound spaced by four base pairs (Fig. 10). The consequence of this intercalation is that the O3'-P religation is structurally impossible because both atoms are too far away from each other (more than 9 Å) (Fig. 10).

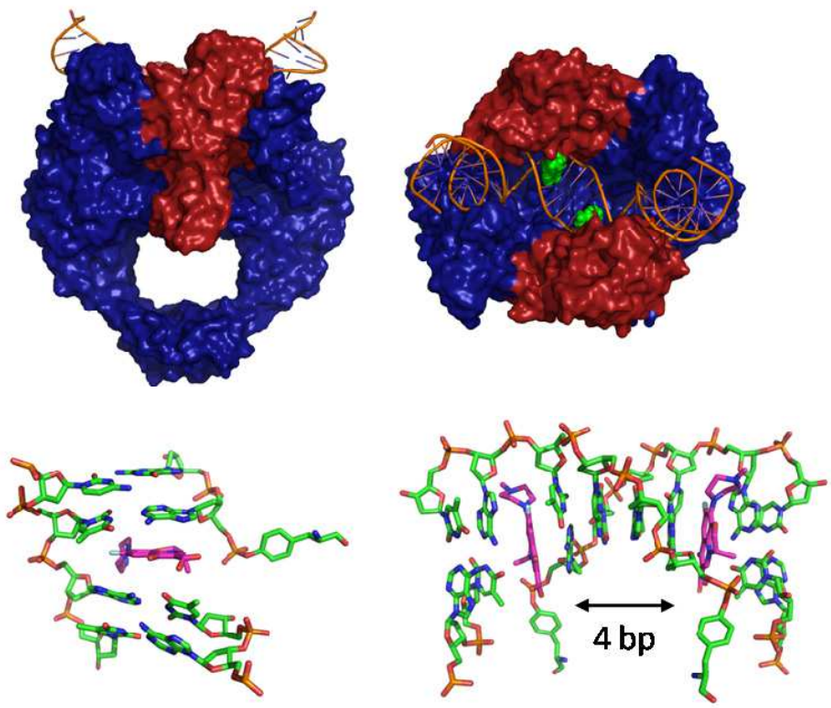

Fig. 10. Structure of the *M. tuberculosis* DNA gyrase catalytic core in complex with DNA and moxifloxacin. Side view (top left) and top view (top right) of the molecular surface of the catalytic core. The Toprim domain is represented in red, the BRD in blue, the 35 base pairs DNA oligonucleotide in orange and the moxifloxacin in green. (Bottom left) Close view of the structure of the intercalated moxifloxacin (magenta) in the broken DNA double helix (green). The catalytic tyrosine (Y129 in the *M. tuberculosis* DNA gyrase sequence) is shown in green outside the DNA helix. (Bottom right) Close view of both moxifloxacin molecules in the broken DNA showing the four base pairs in between the two bound fluoroquinolones. Both catalytic tyrosines of each monomer are shown in green in the DNA major groove.

6. Fluoroquinolone resistance in *M. tuberculosis*

Fluoroquinolone resistance in *M. tuberculosis* is defined by resistance to ofloxacin (ability of the bacilli to grow on medium containing 2 mg/l ofloxacin (WHO, 2001) and is mainly due to mutations in DNA gyrase in the QRDRs (see section 4) (Takiff et al., 1994). Mutations within the QRDRs are responsible of at least 75% of fluoroquinolone resistance in *M. tuberculosis*, affecting most commonly the GyrA subunit (G88A, A90V, S91P, D94G, H, N, Y) but also the GyrB subunit (Aubry et al., 2006b; Veziris et al., 2007; Siddiqi et al., 2002; Feuerriegel et al., 2009). Modeling of the *M. tuberculosis* DNA gyrase catalytic core allowed to clearly establish that QRDR residues of both subunits are spatially close and form the quinolone-binding pocket (QBP) (Fig. 11). In this pocket, the fluoroquinolone is maintained on one side by three residues of the QRDR-A, G88, D89 and A90, that are in close contact with the quinolone conserved carboxylic function (R3 group, Fig. 1 and 11), and on the other side by five residues of the QRDR-B, D500, R521, N538, T539 and E540 in close contact with the R1, R7 and R8 groups of the quinolone (Fig. 1 and 11). Almost all these residues have been shown to be directly involved in the level of resistance to fluoroquinolone when they are modified (Aubry et al., 2004; 2006; Matrat et al., 2006; 2008; Mokrousov et al., 2008; Von Groll et al., 2009; Kim et al., 2011). The model shows that the geometry of the QBP is crucial for the recognition and the stability of fluoroquinolone in the pocket. Any modification of the amino acids belonging to the QBP leads to either a direct or indirect change of the geometry of the pocket (Fig. 11). The pocket becomes too large to stabilize the fluoroquinolone, when the side chain of the modified amino acid becomes smaller. In contrast, the pocket becomes too small to fix the fluoroquinolone, when the side chain of the modified amino acid is bulkier. An indirect effect on the geometry of the pocket is observed when amino acid substitutions are localised in the H4 helix that interacts with the major groove of the DNA. As DNA is also part of the QBP (Fig. 11), modification of the structure of the DNA will modify the geometry of the pocket, and can lead to destabilisation of the fluoroquinolones in the pocket. This mechanism could explain why substitutions of the amino acid D94 (position mostly found in *M. tuberculosis* strains resistant to quinolones) have paradoxical effects, e.g. the substitution by an amino acid with a smaller side chain, such as alanine or glycine, increases the resistance to the same level than the substitutions by amino acids with bulky side chains.

Resistance to fluoroquinolones of *M. tuberculosis* DNA gyrase results from two mechanisms. On one hand, the lower natural affinity of the *M. tuberculosis* DNA gyrase catalytic core for fluoroquinolones is responsible for "intrinsic resistance" and is attributable to the amino acid nature at three positions, 81 and 90 in the QRDR-A and 521 in the QRDR-B (see section 3). On the other hand, DNA gyrase modifications found in the QRDRs of DNA gyrases of clinical *M. tuberculosis* strains are responsible for "acquired resistance". Interestingly, amino acid at two positions in the QBP play a crucial role in both natural and acquired resistance, position 90 in the QRDR-A, which is an alanine in the *M. tuberculosis* sequence and position 521, an arginine in the *M. tuberculosis* QRDR-B. Both amino acid are essential for the shape of the QBP and, as a result, for the binding of quinolone. For example, the substitution of residue A90 in the wild-type *M. tuberculosis* DNA gyrase to S90 generates a pocket better adapted to the size of moxifloxacin (Fig. 12), increasing the susceptibility to this inhibitor. In contrast, the A90V substitution, found in some strains resistant to moxifloxacin, generates a

pocket too small to bind moxifloxacin (Fig. 12). In the QRDR-B, the side chain of residue R521, an amino acid located in the Toprim DBL, points towards the DNA minor groove and forms a "flap" that blocks the fluoroquinolone in the pocket through contact with the R7 group (Fig. 11). The increase of susceptibility to fluoroquinolones by the substitution of R521 to lysine can be explained by the lower energy cost when moving an arginine than a lysine in the DNA minor groove (Rohs et al., 2010). This means that the "flap" created by this amino acid, would find it easier to open up and destabilize the fluoroquinolone, when this residue is an arginine rather than a lysine.

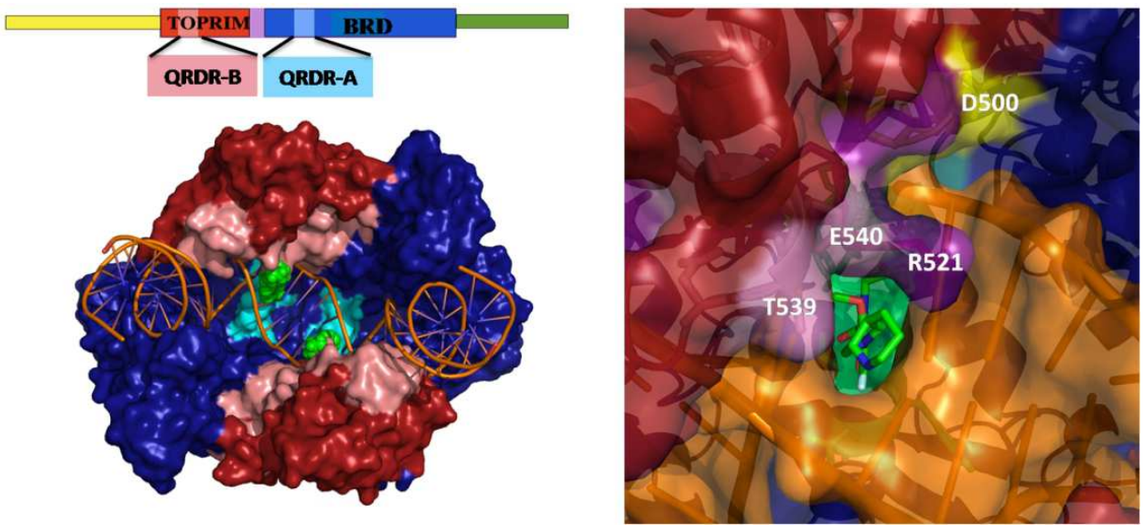

Fig. 11. (Top) Schematic representation of the *M. tuberculosis* DNA gyrase sequence. Subunit A contains the breakage-reunion domain (BRD, blue) and the C-terminal domain (CTD, green). The subunit B contains the ATPase domain (yellow) and the Toprim domain (red). *End of legend*. Localization of the QRDR is indicated in pink and light blue. (Bottom left) Top view of the molecular surface representation of the M. *tuberculosis* DNA gyrase catalytic core in complex with DNA and moxifloxacin showing the QBP (color code is the same than in Fig. 10). The QRDR-A and -B are represented in pink and light blue, respectively. (Right) Close view of the quinolone-binding pocket. The DNA-protein complex is represented in transparent molecular surface and moxifloxacin in sticks (color code is the same than in Fig. 10). The residues of the QRDR-B (Toprim) belonging to the QBP are indicated in pink, purple and yellow. Resides A90 of the QRDR-B is represented in light green in the background of the pocket.

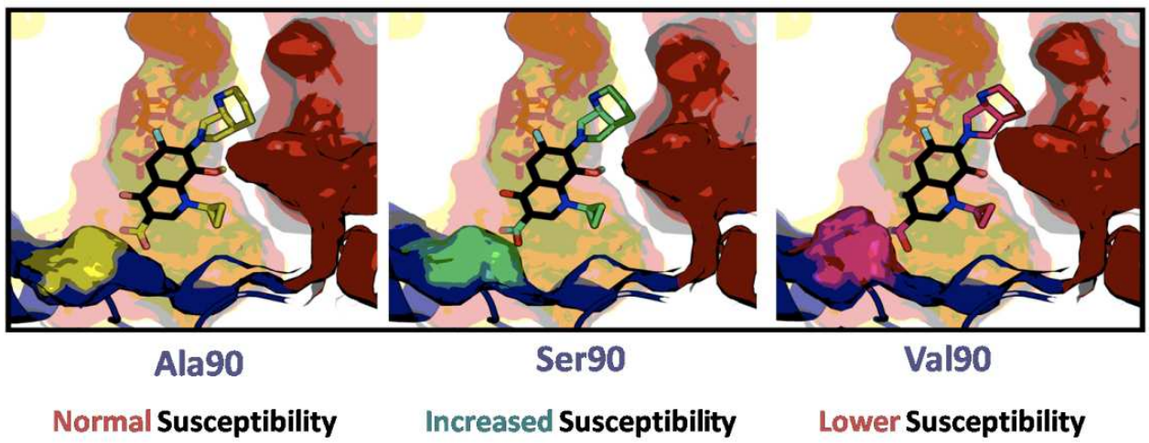

Fig. 12. Effect of the substitution of A90 (QRDR-A) on the geometry of the quinolone-binding pocket. (Left) Quinolone-binding pocket of the wild-type *M. tuberculosis* DNA gyrase. The A90 is coloured in yellow. (Middle) Substitution of A90 to serine (S90 is represented in green). (Right) Substitution of A90 to valine (V90 is represented in magenta).

7. Conclusion

This chapter has described the structural insights into the bacterial type II topoisomerases, the targets of quinolones and into the quinolone resistance mechanism, discussed in the context of the enzyme modifications, e.g. amino acid substitutions, described in the literature that are implicated in quinolone resistance. It has shown that the tridimensional structure of the *M. tuberculosis* catalytic core in complex with DNA and a quinolone molecule is a powerful tool to better understand the relationships between the sequence, the structure and the resistance phenotype in *M. tuberculosis* DNA gyrase.

Knowledge of structural data has applications both in the diagnosis of resistance in *M. tuberculosis* but also in tuberculosis treatment. This powerfulness consists in four essential aspects. It strongly contributes to (1) the analyses of the results obtained by molecular studies since the variety of new mutations, especially in the GyrB subunit, is increasing, (2) the discrimination between substitutions identified in fluoroquinolone-resistant clinical strains that are implicated or not in fluoroquinolone resistance (Pantel et al., 2011), (3) the prediction of the effect on susceptibility to quinolones of new undescribed mutations, and finally (4) to the design of new quinolones, as quinolones used nowadays for tuberculosis treatment have been designed before structural data on the *M. tuberculosis* DNA gyrase catalytic core were available.

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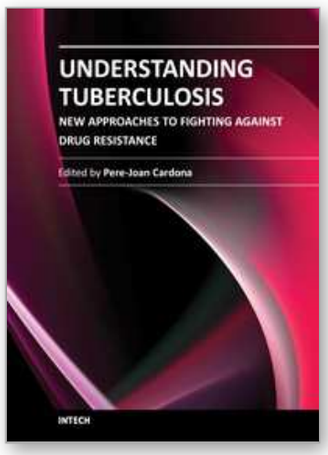

Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance

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In 1957, a Streptomyces strain, the ME/83 (S.mediterranei), was isolated in the Lepetit Research Laboratories from a soil sample collected at a pine arboretum near Saint Raphael, France. This drug was the base for the chemotherapy with Streptomicine. The euphoria generated by the success of this regimen lead to the idea that TB eradication would be possible by the year 2000. Thus, any further drug development against TB was stopped. Unfortunately, the lack of an accurate administration of these drugs originated the irruption of the drug resistance in Mycobacterium tuberculosis. Once the global emergency was declared in 1993, seeking out new drugs became urgent. In this book, diverse authors focus on the development and the activity of the new drug families.

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