Scientific Research

Review: The Molecular Basis of Resistance in *Mycobaterium tuberculosis*

Lorena Cristina Santos

Departamento de Farmácia, Faculdades Objetivo, Goiânia, Brazil
Email: lorenacsantos@yahoo.com.br

ABSTRACT

Tuberculosis is a serious global public health problem and its high prevalence is strongly associated with the increase of drug resistance. This steady increase in the frequency of *M. tuberculosis* strains resistant to one or more agents commonly used to treat tuberculosis has drawn worldwide attention to understanding the molecular basis of resistance in *M. tuberculosis*. TB resistance is a great concern in the antibiotic resistance pandemic due to the high risk of death, as patients can remain infected for months or years and also because of the difficulty of the treatment. A molecular understanding of the series of events that render *M. tuberculosis* multi-drug resistant is very important in order to find a fast and appropriated diagnosis as well as a new target for new drugs.

1. Introduction

Tuberculosis (TB) still persists as a major cause of morbidity and mortality, affecting almost a third of the world's population, remaining one of the biggest public health problems in the 21st century. Although TB is a curable disease, some factors have been identified as reasons for a mounting global TB burden, such as inadequate detection, cure rates, and Aids, resulting in individuals more susceptible to development of TB [1,2]. A steady increase in the frequency of *Mycobacterium tuberculosis* strains resistant to one or more agents commonly used to treat TB including isoniazid (INH), rifampin (RMP), streptomycin (STR), ethambutol (EMB), or pyrazinamide (PZA), components of the first-line multidrug therapy, is also of concern [3].

Chemotherapy for TB consists in a two-stage process, including three or more drugs, beginning with an initial stage of bacterial eradication, followed by a second stage devoted to elimination of more resistant or latent organisms. The necessity of using a combined therapy may be explained by the historical reports of treatment using a single drug resulting in the selection of resistant strains and treatment failure [4]. Additionally, in a mycobacterial population, there are different populations of bacilli, and each can present a different susceptibility profile for anti-TB drugs [5].

Mobile genetics elements such as plasmids and transposons, which are known to mediate drug resistance in various bacterial species, do not do so in *M. tuberculosis* [6]. In a population of *M. tuberculosis*, resistance to anti-TB drugs is due to spontaneous chromosomal mutations that occur at a relatively low frequency, 10^{-6} to 10^{-8} mycobacterial replications. Individual nucleotide changes (point mutations) confer resistance to single drugs, and the sequential accumulation of these mutations in different genes involved results in multi-drug resistance (MDR-TB) [7,8].

According to the World Health Organization (WHO), in order to be considered MDR-TB, the *M. tuberculosis* strain should be resistant to at least RMP and INH [9]. Treating TB in these patients is difficult, as they carry strains resistant to the two most efficient anti-TB drugs. These strains require treatment with second-line drugs that have limited treatment efficacy with more toxicity [3]. Additionally, in the last two decades, with the misuse of other drugs with anti-TB action, in particular the fluoroquinolones (FQs), the most effective among the second-line drugs, resistance has broadened to extensively drug-resistant TB (XDR-TB defined as MDR-TB with additional bacillary resistance to FQs and at least one second-line injectable drug) [9].

Clinically significant drug resistance in TB may develop during anti-TB treatment (acquired resistance). Anti-TB drugs impose selection pressure in a population of *M. tuberculosis* in which resistant mutants gradually outnumber susceptible bacilli and emerge as the dominant

strains. Several factors contribute to this selection pressure, such as: monotherapy due to irregular drug supply, inappropriate doctor prescription and, most importantly, poor patient adherence to treatment [6]. Once resistant bacillary strains emerge during treatment, these could be transmitted in a community. Those who are infected with drug-resistant strains may develop drug-resistant TB prior to treatment (primary resistance) [9,10].

MDR-TB is a great concern in the antibiotic resistance pandemic due to the high risk of death, because patients can remain infected for months or years, and due to the high risk of contracting XDR-TB [3,11]. The Anti-tuberculosis Drug Resistance Surveillance Global Project published by the WHO and the International Union Against Tuberculosis and Lung Disease [9], reviewing the global status of TB, have pointed to an increasing incidence of drug-resistance TB in several countries in the world. The report includes data of 90.726 patients from 83 countries and the median prevalence of MDR-TB in new cases was 1.6% (interquartile range 0.6 - 3.9), ranging from 0%, in eight countries with low TB prevalence, to 19.4% in Moldova and 22.3% in Baku, Azerbaijan. The median of MDR-TB among the patients previously treated was 11.7% (interquartile range 4.9 - 20.9) and the highest proportion of MDR-TB was 60% in Tashkent, Uzbekistan [9,12]. For TB control, monitoring the emergence of drug resistant strains and their early detection is essential for appropriate treatment to prevent the development of further resistance and the spread of resistant strains [1]. Data of XDR-TB were rather incomplete, as 37 countries/territories only (mainly low-prevalence ones) reported representative data on XDR-TB in the period 2002-2007. In total, data was available in3818 MDR-TB cases, of which 304 (8.0%) were XDR-TB [12].

Anti-TB drug resistance, particularly multiresistance, has been a subject of concern worldwide and is the result of the several factors related to programs for the TB control, such as treatment regimen and patient adherence, study population, area endemicity, *M. tuberculosis* strain and others. Consequently, more than 450,000 MDR-TB cases are estimated to occur globally each year [13] and a global surveillance of *M. tuberculosis* drug resistance has been proposed to guide appropriate treatment policies [14]. In the last years, studies have shown that certain emerging *M. tuberculosis* strains, like Beijing/W and Haarlem strain families, induce more severe forms of TB, manifest higher failure/relapse than others, and are strongly associated with drug resistance [15-17]. The present occurrences of these strains in developed countries emphasize TB burden even in countries with good TB control programs, and broaden the horizon for the elimination of this disease.

This steady increase in the frequency of *M. tuberculosis* strains resistant to one or more agents used in treatment and the difficulty of their diagnosis prompted us to review the genetic basis of resistance against main drugs utilized in TB treatment. Firstly, because in order to control the drug resistance epidemic it is necessary to gain insight into how *M. tuberculosis* develops drug resistance. Secondly, because this knowledge will help us to understand how to prevent the occurrence of drug resistance and formulation of rapid and unambiguous strategies for detection of resistant strains, which is currently a slow and tedious process.

2. Isoniazid (INH)

INH, isonicotinic acid hydrazide, is a pro-drug that must be metabolized into isonicotinc acid by mycobacterial catalase-peroxidase hemoprotein (katG), encoded by the *katG* gene. The mode of action of INH has been the subject of intensive studies, but it is known to generate a range of highly reactive compounds, including reactive oxygen species (ROS) such as superoxide, peroxide and hydroxyl radical [18], nitric oxide [19], reactive organic species such as isonicotinic-acyl radical or anion [20], and certain electrophilic species [21], which then attack multiple targets in *M. tuberculosis*. This attack results in pleiotropic effects like the inhibition of protein and nucleic acid synthesis [3,22]. Additionally, INH inhibits InhA, a nicotinamide adenine dinucleotide (NADH)-specific enoylacyl carries protein (ACP) reductase involved in fatty acid synthesis, resulting in loss of acid-fastness, probably as a result of the inhibition of the synthesis of mycolic acids, the long-chained found in the cell wall [23, 24]. However, despite this progress regarding understanding the action of INH, the exact mechanism (s) against *M. tuberculosis* remains to be fully delineated, as multiple tar- gets and pathways have been considered [19].

INH is the most used first-line anti-TB drug, and has been the cornerstone of all affective regimes for the treatments of TB disease and latent infection. *M. tuberculosis* is highly susceptible to INH (minimum inhibitory concentration [MIC] 0.02 - 0.2 μg/ml), having the strongest early bactericidal activity against growing tubercle bacilli, but is not active against non-replicating bacilli or under anaerobic conditions [6]. The isonicotinic acid hydrazide is most capable in preventing the emergence of bacillary resistance to companion drugs, but is usually the first drug that becomes resistant when a 6-month RMP-containing short-course regimen is applied [25].

INH resistance is the most common form of anti-TB drug resistance encountered, whether in isolation or in combination with other drugs [9]. There are still many unexplained points concerning the action of INH on *M. tuberculosis*. Although there are still about 10% to 25% of INH-resistant strains in which neither of the known genes are affected by genetic modifications, it is known the most part of INH resistance is controlled by a complex genetic

system that involves genes such as *katG*, *inhA*, *ahp*C, *kasA*, and *ndh* [3,26]. However, mutations in at least 16 other genes have been reported to be associated with INH resistance in clinical isolates [27].

Between 40% and 95% of INH resistant clinical isolates result from blocking pro-drug activation through deletions or frameshifts in *katG* gene that diminished or altered mycobacterial catalase-peroxidase activity, resulting in high levels of INH resistance [14,28,29]. Most mutations are found in region comprising codons 138 and 328, with the most commonly observed gene alteration (75% to 90% frequency) being at codon 315 of the *katG* gene [30]. The substitution of a single nucleotide at position 315 of *katG* (S315T), vary from 53% to 96% of INH resistant isolates. Beijing families isolated from several countries usually carry a higher percentage of the *katG* S315T shift, compared to non-Beijing family isolates [15,31-33]. These mutations may be favored because mutations at this location appear to decrease INH activation without abolishing catalase-peroxidase activity and, therefore, without diminishing the virulence or transmissibility of *M. tuberculosis* strains [17]. The region of the genome within which *katG* lies appears to be relatively unstable and therefore susceptible to aforementioned alterations, possibly because of the presence of repetitive DNA sequences. This instability may contribute to the relatively high rates of generation of INH-resistant mutants, typically quoted as about 1 in 10^5 - 10^6 organisms during in vitro selection [28].

Although the S315T mutation is the most common, genetic modification at the active site of bacterial catalase-peroxidase enzymes as well as the heme cofactor binding site have resulted in an important effect on enzymatic function and thus isoniazid resistance [34]. Mutations at codons V33Stop, D65E, D94A, G99E, H108E, N138S/H, S140A/N, D142A, L150A, S160L, A172T, T180C, V200Stop, F252L, T262R, P275T, Q294Stop, W299G, W328G, I335T, A350S, in *katG* gene have been also associated with high level of INH resistance [35].

M. tuberculosis may compensate for *katG* mutations by overexpressing the *ahpC* gene, that encodes alkyl-hydroperoxide reductase, protein involved in cellular regulation of oxidative stress capable of detoxifying damaging organic peroxides [17,36]. Five different nucleotide alterations have been identified in the promoter region of *ahpC* which lead to overexpression of this protein in INH resistant isolates: −48(G→A), −51(G→A), −54(C→T), −74(G→A) and −81(C→T) [37]. Additionally, mutations within the *oxyR-ahpC* intergenic region that result in increased expression of alkyl hydroperoxidase reductase are also considered to compensate for peroxide sensitivity due to loss of katG function found in clinical resistant strains [38,39]. Nevertheless, the correlation between polymorphic sites in the *oxyR-ahpC* region with INH

resistance in *M. tuberculosis* requires further examination.

INH resistance may still be developed through alterations or overexpression of the INH drug target InhA that is encoded in an operon formed by *inhA* and *mabA* genes. The *inhA* (ORF) gene encode an enoyl acyl carrier protein reductase which plays a role in the synthesis of mycolic acids, and it seems that a toxic derivative of INH inhibits directly the InhA protein binding to the InhA-NADH complex to form a ternary complex that results in inhibition of mycolic acid biosynthesis [40]. Six point mutations associated with INH resistance within the structural *inhA* gene have been identified (I16T, I21T, I21V, I47T, V78A and I95P), although these mutations in the structural *inhA* gene are associated with INH resistance, it is known that just about 0% to 5% of INH *M. tuberculosis* clinical isolates have mutations at the ORF region [39,41]. *InhA* promoter mutations are more frequently seen, about 8% to 20%, and are present at positions −24(G-T), −16(A-G), or −8(T-G/A) and −15(C-T) [41]. This *inhA* promoter gene region regulates the expression of the enoyl acyl carrier protein reductase and those mutations may increase the level of protein expression [17,30,41,42]. The overexpression of *inhA*, due to mutations in the *inhA* promoter region, leads to low level INH resistance and is accompanied by cross-resistance to the second-line anti-TB drug ethionamide (ETH) which has structural similarity to that of isoniazid [43].

Recently two other genes also involved in INH resistance, *kasA* and *ndh*, were found, but with less frequency. The first encodes a *β*-ketoacyl-ACP synthase (KasA) protein involved in the synthesis of meromycolic acids [44] and its overexpression leads to resistance to INH at a MIC of 0.1 μg/ml [45]. Genotypic analysis of the *kasA* gene revealed 4 different amino acid substitutions involving codon 66 (GAT→AAT), codon 121 (AGG→AAG), codon 269 (GGT→AGT), codon 312 (GGC→AGC), codon 387 (GGC→GAG) and codon 413 (TTC→TTA) [44,46]. Although similar mutations were also found in INH susceptible isolates [47], the possibility of *kasA* constituting an additional resistance mechanism should not be excluded [41].

Mutations in *ndh*, a gene encoding an NADH dehydrogenase that is bound to the active site of *inhA* to form the ternary complex with activated INH [48], were recently found to confer resistance to INH and ETH in *M. bovis*. The *ndh* mutants had altered NADH/NAD ratios, causing defects in the enzymatic activity. Thus, defects in the oxidation of NADH to NAD results in NADH accumulation and NAD depletion [48], which appears to protect them from INH-mediated toxicity, since these high levels of NADH can then inhibit the binding of the INH-NAD adduct to the active site of the InhA enzyme [30,48,49]. About 9.5% of INH resistant samples showed point mutations in the *ndh* gene at codons 110 and 168 and

these mutations were not detectable in the INH susceptible group [48].

Studies of mutations associated with INH resistance have been limited because of the variety of genes involved; the number of isolates evaluated; and absence of correlation with *in vitro* INH levels determined by minimal inhibitory concentration. Dalla Costa and colleagues [17] characterized mutations in *katG*, *ahpC* and *inhA* (ORF and regulatory regions) gene loci from 224 INH resistant *M. tuberculosis* isolates in countries in South America. Among these, the *katG* gene was the most frequently mutated (80.8%; 181/224). The mutation in codon 315 of the *katG* gene was present in 178 isolates, comparable to the previously reported rate for patients diagnosed in Kuwait, Brazil and The Netherlands (65% and 55%, respectively), but lower than described in Russia [32,50-52]. Mutations in *inhA* regulatory region were the second most frequent (9.8%), following by *ahpC* gene (8.9%), and *inhA* ORF region (1.3%).

3. Rifampin (RMP)

RNA polymerase (RNAP) is a crucial enzyme in the transcriptional process and is the final target for regulatory pathways controlling gene expression in all living organisms. In bacteria, RNAP is responsible for synthesizing mRNA, rRNA and tRNA [53]. The 400 KDa RNAP core enzyme consists of five different subunits, including an α-dimer (α_2), β subunit, β' subunit and ω subunit. These subunits are converted to a holoenzyme following the binding of one σ subunit, which initiates transcription from promoters [54]. The genes encoding subunits α, β, β', ω and σ have been designated *rpoA*, *rpoB*, *rpoC*, *rpoZ* and *rpoD*, respectively [54,55].

RMP was introduced for use in anti-tuberculosis therapy in the early 1970s and is a very important first line drug treatment of TB. RMP is bactericidal because inhibits the essential *rpoB* gene product β-subunit of DNA-dependent RNA polymerase activity of bacterial but not of mammalian origin, acting early in transcription. It is thought to bind to the β subunit, close to the RNA/DNA channel, and physically blocks the elongation of the growing RNA chain after 2 - 3 nucleotides have been added. In *M. tuberculosis* the bactericidal action has been identified from generating of apoptosis via activation of the "suicide gene module" *mazEF* [56].

RMP is one of the most potent anti-tuberculosis drugs. More than 90% of RMP-resistant TB-causing isolates are also resistant to INH, and RMP resistance is therefore a valuable surrogate marker for MDR-TB [39]. RMP is bactericidal for *M. tuberculosis*, and is active against both growing and stationary phase bacilli with low metabolic activity, with MICs ranging from 0.05 to 1 μg/ml on solid or liquid media, but the MICs is higher in egg media (MIC = 2.5 - 10 μg/ml). Strains with MICs< 1 μg/ml in liquid or

agar medium or MICs< 40 μg/ml in Lowenstein-Jensen (LJ) medium are considered RMP-susceptible [57].

Resistance to RMP occurs at a low frequency, 10^{-7} to 10^{-8}. Because of this , RMP monoresistance in *M. tuberculosis* is rare, except perhaps in HIV-infected patients [58]. Extensive studies on the *rpoB* gene in RMP resistant isolates of *M. tuberculosis* identified a variety of mutations and short deletions in the gene. Since 1993, when Telenti and colleagues [59] cloned and sequenced the core region of the *M. tuberculosis rpoB* gene, a large number of mutations have been reported from strains isolated in many countries worldwide [60-64]. As in other bacteria, mutations in a small defined hot-spot region of the 81 base pair (bp) of the *rpoB* gene (codons 432 to 458 in *M. tuberculosis* and 507 to 533 in *Escherichia coli*, named cluster I), termed the rifampicin-resistance determining region (RRDR), are found in about 94% - 98% of RMP-resistant *M. tuberculosis* isolates [3,65]. About 69 single nucleotide changes; 3 insertions, 16 deletions and 38 multiple nucleotide changes have been reported in this region [66]. However, Herrera *et al.* [66] and other authors [60,61,63] have detected mutations, associated with RMP resistance, outside the 81-bp-core region, such as at the codons 481, 490, 498, 505, 534, 535, 553, 561, 571, 572, 633, and 672, although less frequently.

Both clinical and laboratory derived mutants are seen around amino acids 513-531, generally resulting in high level resistance (MIC< 32 μg/ml) [56,67,68]. Mutations at positions 531, 526 and 516 are among the most frequent mutations in RMP-resistant strains [68]. Siddiqi and colleagues [69], analyzed 93 RMP-resistant isolates from India, of these, 28 had missense mutation S531L and 8 had the substitution S531W. The next most common mutations among those isolates were the amino acid substitutions D516V or D516G (20 isolates) and H526Y, H526L, or H526R (19 isolates). In this study, the amino acids 526 to 531 appear to be very important in drug target interactions, and mutations in them result in MICs in the range of 64 μg/ml and above.

However, the frequency and nature of mutations in the *rpo*B gene among RMP-resistant *M. tuberculosis* strains vary considerably according to the geographical location or the ethnic populations [70-72]. Kapur and colleagues [73], examined US strains collection and found strains with a CAC→TAC codon 526 change (H→Y) accounted for about 30%, while organisms with this mutation represented only 12% of the samples from nine countries studied by Telenti *et al.* [67]. In addition, mutations in codon 533 have been reported for RMP-resistant and RMP-susceptible strains by Taniguchi *et al.* [74]. However, in studies conducted with isolates from same regions in Brazil and France, this mutation was strongly involved with RMP resistance [75].

4. Pyrazinamide (PZA)

PZA is an important first-line drug used along with INH and RMP and plays an essential role in TB treatment because it kills a population of latent bacilli in acidic pH environment in lesions which is not killed by other drugs [57]. PZA has high sterilizing activity in vivo [76], but no activity against tubercle bacilli at normal culture conditions near neutral pH, since PZA is only active against *M. tuberculosis* at acid pH (e.g. 5.5) [6]. Even at acid pH, PZA activity is quite poor, with MICs in the range of 6.25 - 50 μg/ml. PZA activity is enhanced under low oxygen or anaerobic conditions [77] and by agents that compromise the energy status, such as weak acids [78], and energy inhibitors such as DCCD (dicyclohexylcarbodiimide), azide and rotenone [76].

The mechanism of action of PZA is poorly understood. It is known that PZA is a pro-drug that enters the organism through passive diffusion and requires conversion to its active form, pyrazinoic acid (POA), by the pyrazinamidase/nicotinamidase enzyme encoded by *pncA* gene of *M. tuberculosis* [79]. PZA-susceptible *M. tuberculosis* isolates possess a pyrazinamidase (PZAse) that is constitutively expressed and hydrolyzes PZA to POA, which is the lethal molecule inhibiting various functions at acid pH in *M. tuberculosis* [80]. The POA produced intracellularly reaches the cell surfaces through passive diffusion and a defective efflux [81]. The accumulation of POA and protonated POA lowers the intracellular pH to a suboptimal level that may inactivate many pathways, including fatty acid synthase and membrane transport function [82]. The protonated POA brings protons into cell and could eventually cause cytoplasmic acidification and de-energize the membrane by collapsing the proton motive force, which affects membrane transport [76]. The target of PZA is related to membrane energy metabolism, although it is widely accepted that POA may not have a specific target, but rather that cellular acidification causes inhibition of major processes [82].

PZA-resistant *M. tuberculosis* strains lack PZAse activity and mutations in the *pncA* gene is known to be associated with this resistance [83-85]. Several authors have reported that 72% to 95% of PZA-resistant clinical isolates of *M. tuberculosis* carried *pncA* mutations and these mutations are unusually located spread throughout the gene, although there are three areas of clustered mutations around amino acids 3 - 71, 61 - 85 and 132 - 142 [82,86]. Studies have reported that the alignment of the amino acid sequences of PZAses from various species reveals that this region contains highly conserved residues, supporting the idea that this region should be structurally and/or catalytically important for the PZAse activity [87,88]. A small number of PZA mutations occur outside the *pncA* gene but these have not been characterized [86].

5. Ethambutol (EMB)

EMB [(+)-2,2'-(ethylenediimino)di-1-butanol] is one of the primary drugs used in combination with INH, RMP and PZA to prevent the emergence of drug resistance. EMB is a bacteriostatic agent that is active for growing bacilli and has no effect on non-replicating bacilli [89]. Although several hypotheses have been proposed to explain the mechanism of action of EMB, most studies have implicated a detrimental alteration of the mycobaterial cell wall structure [90] through inhibiting the polymerization of cell wall arabinan of arabinogalactan and of lipoarabinomannan, and induces the accumulation of D-arabinofuranosyl-P-decaprenol, an intermediate in arabinan biosynthesis [84,90]. Arabinosyl transferase, encoded by *embB*, an enzyme involved in the synthesis of arabinogalactan, has been proposed as the target of EMB in *M. tuberculosis* and *M. avium* [91]. In *M. tuberculosis*, *embB* is organized into a 10-kb operon with *embC* and *embA* genes named *embCAB* [92]. Mutations in *embCAB* operon, in particular *embB*, are responsible for EMB resistance. Mutations at *embCAB* resulted in MICs of 7.5 - 50 μg/ml and mutations at codon 306 in *embB* is one of the most common (>68%) in *M. tuberculosis,* making it the ethambutol resistance determining region (ERDR) [93], in particular M306, which is often replaced by isoleucine, leucine or valine [94,95]. However, about 35% of EMB-resistant strains do not have *embB* mutations [96], suggesting that there may be other mechanisms of EMB resistance.

Studies conducted by Sreevatsan and colleagues [90] comparing EMB-resistant versus EMB susceptible *M. tuberculosis* strains showed there is restricted variation in *embCAB* in natural populations of *M. tuberculosis* recovered from diverse geographic sources and there is a unique and common association of EmbB amino acid residue 306 substitutions in EMB resistance. They found five distinct mutant codons resulting in three different amino acid replacements at EmbB position 306 of EMB-resistant organisms and concluded that these mutants have arisen by positive Darwinian selection in the course of drug therapy.

6. Streptomycin (STR)

STR is an aminocyclitol glycoside utilized as alternative first line anti-TB drug recommended by the WHO [9]. The effect of STR has been demonstrated to take place at the ribosomal level, having activity against a variety of bacterial species [67]. The STR binds to the 30S subunit of bacterial ribosome interacting with the 16S rRNA and S12 ribosomal protein (*rrs* and *rpsL* genes, respectively) [97], inducing ribosomal changes, which cause misreading of the mRNA and inhibition of protein synthesis [98]. STR kills actively growing tubercle bacilli with MICs of 2

- 4 μg/ml [99], but it is inactive against non-growing or intracellular bacilli [57].

STR is the least toxic of the aminoglycosides utilized for TB, but resistance development is unacceptably rapid [100]. *M. tuberculosis* becomes resistant when targets of STR in the ribosomes are mutated. Point mutations in STR resistant isolates have been reported to both *rpsL* and *rrs* genes, and these are the major mechanisms of STR resistance, accounting for 65% - 67% of STR-resistant strains [39,101,102]. The most frequently observed mutations in *rpsL* gene are at codon 43 (AAG→AGG/ACG; K→R/T) and at codon 88 (AAG→AGG/CAG; K→R/Q), being reported in 50% of STR-resistant strains [102]. *M. tuberculosis* has only a single copy of the *rrs* gene, which encode the 16S rRNA. Thus, the loop of 16S rRNA that interact with S12 protein constitute an easily selected mutation site [84,101]. The 530 loop region is part of the aminoacyl-tRNA binding site and is involved in the decoding process. Mutations are clustered in this region as well as in the adjacent 915 region [103]. About 20% of STR resistant has a C→T transition at positions 491, 512 and 516, and a A→C/T transition at positions 513 in that highly conserved 530 loop [39,103].

MIC analysis of STR resistant isolates indicate that amino acid replacements in the *rpsL* genes correlate with a high level of resistance, whereas mutations in the *rrs* gene correlate with an intermediate level [104] and altered cell permeability or mutations which lie outside of this genes have been related with low level of STR resistance [41].

7. Second Line Drugs

RMP, EMB and PZA—containing short-course chemotherapy remains efficacious in the treatment of INH resistant strains, but its efficacy becomes substantially compromised in the treatment of MDR-TB, denoted by bacillary resistance to at least both INH and RMP. However, MDR-TB is not incurable. According to the WHO, for those strains that are MDR-TB, second line drugs must be used, such as: aminoglycosides (kanamycin and amikacin), polypeptides (capreomycin, viomycin and enviomycin), fluoroquinolones (FQs-ofloxacin, ciprofloxacin, and gatifloxacin), D-cycloserine and thionamides (ethionamide and prothionamide). Unfortunately, these second-line drugs are inherently more toxic and less effective than first-line drugs [9] and in March 2006, a report jointly published by the US Centers for Disease Control and Prevention and the WHO described a severe form of disease, XDR-TB (extensively drug resistance TB) [105], presently defined as MDR-TB, with additional bacillary resistance to any FQs and at least one of second-line injectable drugs [106]. The phenotypic methods to detect resistance to second line drugs are less well established and the molecular mechanisms of resistance are also less

defined [41].

Levofloxacin (LVF) and ofloxacin (OFL) are the two FQs used as second-line drugs in MDR-TB treatment [9]. FQs inhibits DNA gyrase (topoisomerase II) and topoisomerase IV, two essential enzymes responsible for maintaining chromosomes in an appropriated topological state, resulting in microbial death [107]. DNA gyrase is a tetrameric A2B2 protein. The A subunit carries the breakage-reunion active site, whereas the B subunit promotes adenosine triphosphate hydrolysis. *M. tuberculosis* has *gyrA* and *gyrB* encoding the A and B subunits, respectively, which has conserved region, the quinolone-resistance-determining region (QRDR) with 320 bp and 375 bp respectively. These are points of interaction of FQ and gyrase [108], and mutations in *gyrA* are associated with high-level resistance while *gyrB* mutations have been just identified in laboratory mutants of *M. tuberculosis* [84]. However, the percentage of FQs resistants *M. tuberculosis* clinical isolates with detectable *gyr* mutations may vary from 2% to 100% among different studies [109-112]. This variance can be explained by the differences in the extension of coverage of the genome [108]; the definition of MICs [111]; and maybe, others mechanisms responsible for the mycobacterial resistance to FQs, such as decrease in cell-wall permeability to drug, drug efflux pump, drug sequestration, or perhaps even drug activation [6,107]. In the last years, a new mechanism of FQs resistance related with MfpA was reported, a member of the pentapeptide repeat family of proteins from *M. tuberculosis,* by binding to DNA gyrase and inhibition of its activity [113]. However, this alternative mechanism related to lower levels of resistance, unlike that due to *gyr* mutations [6,111].

Kanamycin (KM) and its derivative amikacin (AMK), are also aminoglycoside inhibitors of protein synthesis, like STR, through modification of ribosomal structures at the 16S rRNA and cannot be used against dormant bacillus [114]. Aminoglycosides bind to bacterial ribosomes and disturb the elongation of the peptide chain in the bacteria [115]. Ribosomal changes in the 16S rRNA lead to possible cross-resistance with STR, KM and AMK, but this is not always complete, because KM and AMK were still efficacious *in vitro* when resistance to STR had developed [116]. Mutations at positions 1400, 1401 and 1483 of the *rrs* gene (16S rRNA) are associated with resistance to KM and AMK [115].

Viomycin (VM) and capreomycin (CPM) are polypeptide antibiotics whose mode of action is not fully understood, but it is known that they inhibit prokaryotic protein synthesis and are also used as second-line anti-TB drugs. As these drugs have such potent activity against the persistent forms of TB, they may have a target or secondary target outside the ribosome [117]. The rRNA methyltransferase, encoded by *tlyA* gene, was shown to be involved in resistance to CPM and VM [118]. This rRNA

methyltransferase modifies nucleotide C1409 in helix 44 of 16S rRNA and nucleotide C1920 in helix 69 of 23S rRNA [119]. Additionally, mutations in the *rrs* gene that encodes the 16S rRNA are also associated with resistance to VM and CPM, specifically a G→T nucleotide changes at codon 1484 [120,121]. Cross-resistance can be found between KM, AMK, CPM and VM [6].

Ethionamide (ETH) is an important drug in the treatment of MDR-TB and is mechanistically and structurally analogous to INH. Like INH, ETH is also thought to be a prodrug [6]. However, ETH is activated by a *katG*-independent mechanism leading to the forming of an S-oxide metabolite that has considerably more activity than the parent drug. Same studies have reported that *ethA* (also called *etaA*), which codes for a flavin mono-oxygenase, is responsible for the activation of ETH [122-124]. The mode of action of the activated form of ETH is via inhibition of the *inhA* gene product enoyl-ACP reductase [21,42]. The activated drug disrupts cell wall biosynthesis by inhibiting mycolic acid synthesis [125]. Mutations in the promoter of the *inhA* and *ethA* genes are associated with resistance to ETH [124,125]. As the enzymes responsible for ETH activation is different from INH activation, only mutants derived from alterations of the gene *inhA* do show cross-resistance between INH and ETH [122].

D-cycloserine (DCS) is a cyclic analog of amino acid D-alanine, which is one of the central molecules of the cross linking step of peptideoglycan assembly [39]. DCS inhibits alanine racemase (Alr) and D-alanine: D-alanine ligase (Ddl) which synthesizes the pentapeptide core using D-alanine; both enzymes are essential in the synthesis of peptidoglycan and subsequently in cell-wall biosynthesis and maintenance [126]. Over expression of *alr*, specifically due to a G→T transversion in the promoter region, cause DCS resistance [39].

8. Conclusions

According to Cohen *et al.* [127], there are three determinants that influence the proportion of drug-resistant TB among all cases in a population-based study. First, the size of the subpopulations infected with susceptible strains and the probability of developing TB among those infected with susceptible strains. Second, the size of the subpopulation infected with drug-resistant strains and the probability of developing TB among those infected with resistant strains. Both are related to the risk of infection as well as the immunity of the infection population in containing the infection; the least determinant, strongly associated with the interaction between the bacillary population and the treatment regimen, is the emergence of drug-resistant strains during the treatment among TB patients initially infected with susceptible strains, and with the amplification of drug resistance among those

initially infected with resistant strains.

A real molecular understanding of the series of events that render *M. tuberculosis* multi-drug resistant remains elusive. However, for TB control, monitoring the emergence of drug resistant strains is essential. While detection of drug resistance by phenotype is hindered by the prolonged time to identify resistant strains, genotypic prediction of drug resistance is faster [1].

It is necessary to efficiently interrupt the MDR and XDR-TB ongoing transmission through early diagnosis and effective treatment, which should be a priority target for TB control. Several options of rapid testing of anti-TB drug resistance are available, including DNA sequencing [6], solid-phase hybridization techniques [128], microscopic observation drug susceptibility assay [129], real-time PCR techniques, microarrays [130], slide DST [131], phage-based assays [132], colorimetric methods [133] and nitrate reductase assay [134]. Additionally, RMP resistance has been proposed as molecular marker for MDR-TB. Detecting RMP resistance as a screening strategy in the diagnosis of MDR-TB needs to be tested in settings with a low risk of non-MDR RMP resistance [135].

Furthermore, the diagnosis of latent TB infection specifically caused by drug-resistant strains is currently not possible. Yet, early diagnosis and exact identification of drug resistance during *M. tuberculosis* latency could have a substantial impact on TB control [135]. These and several others factors must be reviewed and analyzed to find a fast and effective means of controlling TB and the drug-resistant strains emergency. The molecular techniques may be the most promissory tools for this purpose.

REFERENCES

[1]   G. S. Kibiki, B. Mulder, W. M. Dolmans, *et al.*, "*M. tuberculosis* Genotypic Diversity and Drug Susceptibility Pattern in HIV-Infected and Non-HIV-Infected Patients in Northern Tanzania," *BMC Microbiol*, Vol. 7**,** 2007, p. 51. doi:10.1186/1471-2180-7-51

[2]   I. M. Baptista, M. C. Oelemann, *et al.*, "Drug Resistance and Genotypes of Strains of *Mycobacterium tuberculosis* Isolated from Human Immunodeficiency Virus-Infected and Non-Infected Tuberculosis Patients in Bauru, Sao Paulo, Brazil," *Memorias do Instituto Oswaldo Cruz*, Vol. 97, No. 8, 2002, pp. 1147-1152. doi:10.1590/S0074-02762002000800015

[3]   L. C. Santos, H. de M. Bousquet, A. M. Pereira, *et al.*, "A High Prevalence of Resistance in New Tuberculosis Cases of Midwestern Brazil," *Infection Genetics Evolution*, Vol. 10, No. 7, 2010, pp. 1052-1057. doi:10.1016/j.meegid.2010.06.018

[4]   P. Silva and J. A. Ainsa, "Drugs and Drug Interactions," In: J. C. Palomino, S. C. Leão and V. Ritacco, Eds., *Tuberculosis. From Basic Science to Patient Care*, 2007. http://www.TuberculosisTextbook.com

[5]   I. C. Shamputa, L. Rigouts, L. A. Eyongeta, *et al.*, "Ge-

notypic and Phenotypic Heterogeneity among *Mycobacterium tuberculosis* Isolates from Pulmonary Tuberculosis Patients," *Journal of Clinical Microbiology*, Vol. 42, No. 12, 2004, pp. 5528-5536. doi:10.1128/JCM.42.12.5528-5536.2004

[6] Y. Zhang and W. W. Yew, "Mechanisms of Drug Resistance in *Mycobacterium tuberculosis*," *The International Journal of Tuberculosis and Lung Disease*, Vol. 13, No. 11, 2009, pp. 1320-1330.

[7] C. E. Ozturk, A. Sanic, *et al.*, "Molecular Analysis of Isoniazid, Rifampin and Streptomycin Resistance in *Mycobacterium tuberculosis* Isolates from Patients with Tuberculosis in Duzce, Turkey," *Japanese Journal of Infectious Diseases*, Vol. 58, No. 5, 2005, pp. 309-312.

[8] H. Soini and J. M. Musser, "Molecular Diagnosis of Mycobacteria," *Clinical Chemistry*, Vol. 47, No. 5, 2001, pp. 809-814.

[9] WHO, "Anti-Tuberculosis Drug Resistance in the World, Fourth Global Report," Word Health Organization, Geneva, 2008.

[10] A. Faustini, A. J. Hall and C. A. Perucci, "Risk Factors for Multidrug Resistant Tuberculosis in Europe: A Systematic Review," *Thorax*, Vol. 61, No. 2, 2006, pp. 158-163. doi:10.1136/thx.2005.045963

[11] A. R. Valim, L. G. Possuelo, *et al.*, "Evaluation and Genotyping of Multidrug-Resistant Cases of Tuberculosis in Southern Brazil," *Microbial Drug Resistance*, Vol. 12, No. 3, 2006, pp. 186-191. doi:10.1089/mdr.2006.12.186

[12] A. Wright, M. Zignol, *et al.*, "Epidemiology of Antituberculosis Drug Resistance 2002-07: An Updated Analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance," *The Lancet*, Vol. 373, No. 9678, 2009, pp. 1861-1873. doi:10.1016/S0140-6736(09)60331-7

[13] K. Weyer, "Survey of Tuberculosis Drug Resistance, 2001-2002," Western Cape, Pretoria, 2003.

[14] T. Cohen, M. C. Becerra and M. B. Murray, "Isoniazid Resistance and the Future of Drug-Resistant Tuberculosis," *Microbial Drug Resistance*, Vol. 10, No. 4, 2004, pp. 280-285. doi:10.1089/mdr.2004.10.280

[15] G. L. Li, D. F. Zhao, *et al.*, "Molecular Characterization of Drug-Resistant Beijing Family Isolates of *Mycobacterium tuberculosis* from Tianjin, China," *Biomedical and Environmental Sciences*, Vol. 23, No. 3, 2010, pp. 188-193. doi:10.1016/S0895-3988(10)60051-7

[16] B. J. Marais, T. C. Victor, A. C. Hesseling, *et al.*, "Beijing and Haarlem Genotypes Are Overrepresented among Children with Drug-Resistant Tuberculosis in the Western Cape Province of South Africa," *Journal of Clinical Microbiology*, Vol. 44, No. 10, 2006, pp. 3539-3543. doi:10.1128/JCM.01291-06

[17] E. R. Dalla Costa, M. O. Ribeiro, *et al.*, "Correlations of Mutations in *katG*, *oxyR-ahpC* and *inhA* Genes and *in Vitro* Susceptibility in *Mycobacterium tuberculosis* Clinical Strains Segregated by Spoligotype Families from Tuberculosis Prevalent Countries in South America," *BMC Microbiology*, Vol. 9, 2009, p. 39. doi:10.1186/1471-2180-9-39

[18] H. A. Shoeb, B. U. Bowman, *et al.*, "Peroxidase-Mediated Oxidation of Isoniazid," *Antimicrobial Agents and Chemotherapy*, Vol. 27, No. 3, 1985, pp. 399-403.

[19] G. S. Timmins, S. Master, *et al.*, "Nitric Oxide Generated from Isoniazid Activation by KatG: Source of Nitric Oxide and Activity Against *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 48, No. 8, 2004, pp. 3006-3009. doi:10.1128/AAC.48.8.3006-3009.2004

[20] R. Rawat, A. Whitty and P. Tonge, "The Isoniazid-NAD Adduct Is a Slow, Tight-Binding Inhibitor of InhA, the *Mycobacterium tuberculosis* Enoyl Reductase: Adduct Affinity and Drug Resistance," *Proceeding of the National Academy of Sciences of the Unite States of Aaerica*, Vol. 100, No. 24, 2003, pp. 13881-13886.

[21] K. Johnsson, D. King and P. Schultz, "Studies on the Mechanism of Action of Isoniazid and Ethionamide in the Chemotherapy of Tuberculosis," *Journal of the American Chemical Society*, Vol. 117, No. 17, 1995, pp. 5009-5010. doi:10.1021/ja00122a038

[22] M. Tsukamura and S. Tsukamura, "Isotopic Studies on the Effect of Isoniazid on Protein Synthesis of Mycobacteria," *Japanese Journal of Tuberculosis and Chest Diseases*, Vol. 11, 1963, pp. 14-27.

[23] K. Takayama, L. Wang and H. L. David, "Effect of Isoniazid on the *in Vivo* Mycolic Acid Synthesis, Cell Growth, and Viability of *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 2, No. 1, 1972, pp. 29-35.

[24] Global and A. TB, "Isoniazid," *Tuberculosis* (*Edinb*), Vol. 88, No. 2, 2008, pp. 112-116. doi:10.1016/S1472-9792(08)70011-8

[25] T. Yoshiyama, H. Yanai, *et al.*, "Development of Acquired Drug Resistance in Recurrent Tuberculosis Patients with Various Previous Treatment Outcomes," *The International Journal of Tuberculosis and Lung Disease*, Vol. 8, No. 1, 2004, pp. 31-38.

[26] V. Valcheva, I. Mokrousov, *et al.*, "Molecular Snapshot of Drug-Resistant and Drug-Susceptible *Mycobacterium tuberculosis* Strains Circulating in Bulgaria," *Infection, Genetics and Evolution*, Vol. 8, No. 5, 2008, pp. 657-663. doi:10.1016/j.meegid.2008.06.006

[27] S. V. Ramaswamy, R. Reich, *et al.*, "Single Nucleotide PolymorPhisms in Genes Associated with Isoniazid Resistance in *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 47, No. 4, 2003, pp. 1241-1250. doi:10.1128/AAC.47.4.1241-1250.2003

[28] R. A. Slayden and C. E. Barry, "The Genetics and Bio-Chemistry of Isoniazid Resistance in *Mycobacterium tuberculosis*," *Microbes and Infection*, Vol. 2, No. 6, 2000, pp. 659-669. doi:10.1016/S1286-4579(00)00359-2

[29] D. A. Rouse and S. L. Morris, "Molecular Mechanisms of Isoniazid Resistance in *Mycobacterium tuberculosis* and *Mycobacterium bovis*," *Infection and Immunity*, Vol. 63, No. 4, 1995, pp. 1427-1433.

[30] M. H. Hazbon, *et al.*, "Population Genetics Study of Isoniazid Resistance Mutations and Evolution of Mul-

tidrug-Resistant *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 50, No. 8, 2006, pp. 2640-2649. doi:10.1128/AAC.00112-06

[31] Y. K. Park, *et al.*, "Comparison of Drug Resistance Genotypes between Beijing and Non-Beijing Family Strains of *Mycobacterium tuberculosis* in Korea," *Journal of Microbiological Methods*, Vol. 63, No. 2, 2005, pp. 165-172. doi:10.1016/j.mimet.2005.03.002

[32] M. Y. Lipin, *et al.*, "Association of Specific Mutations in *katG*, *rpoB*, *rpsL* and *rrs* Genes with Spoligotypes of Multidrug-Resistant *Mycobacterium tuberculosis* Isolates in Russia," *Clinical Microbiology and Infection*, Vol. 13, No. 6, 2007, pp. 620-626. doi:10.1111/j.1469-0691.2007.01711.x

[33] I. Mokrousov, *et al.*, "High Prevalence of KatG Ser315Thr Substitution among Isoniazid-Resistant *Mycobacterium tuberculosis* Clinical Isolates from Northwestern Russia, 1996 to 2001," *Antimicrobial Agents and Chemotherapy*, Vol. 46, No. 5, 2002, pp. 1417-1424. doi:10.1128/AAC.46.5.1417-1424.2002

[34] B. C. Finzel, T. L. Poulos and J. Kraut, "Crystal Structure of Yeast Cytochrome C Peroxidase Refined at 1.7-A Resolution," *Journal of Biological Chemistry*, Vol. 259, No. 21, 1984, pp. 13027-13036.

[35] B. Heym, B. Saint-Joanis and S. T. Cole, "The Molecular Basis of Isoniazid Resistance in *Mycobacterium tuberculosis*," *Tubercle and Lung Disease*, Vol. 79, No. 4, 1999, pp. 267-271. doi:10.1054/tuld.1998.0208

[36] D. R. Sherman, *et al.*, "Compensatory *ahpC* Gene Expression in Isoniazid-Resistant *Mycobacterium tuberculosis*," *Science*, Vol. 272, No. 5268, 1996, pp. 1641-1643. doi:10.1126/science.272.5268.1641

[37] A. Telenti, *et al.*, "Genotypic Assessment of Isoniazid and Rifampin Resistance in *Mycobacterium tuberculosis*: A Blind Study at Reference Laboratory Level," *Journal of Clinical Microbiology*, Vol. 35, No. 3, 1997, pp. 719-723.

[38] M. Zhang, *et al.*, "Detection of Mutations Associated with Isoniazid Resistance in *Mycobacterium tuberculosis* Isolates from China," *Journal of Clinical Microbiology*, Vol. 43, No. 11, 2005, pp. 5477-5482. doi:10.1128/JCM.43.11.5477-5482.2005

[39] S. Ramaswamy and J. M. Musser, "Molecular Genetic Basis of Antimicrobial Agent Resistance in *Mycobacterium tuberculosis*: 1998 Update," *Tubercle and Lung Disease*, Vol. 79, No. 1, 1998, pp. 3-29. doi:10.1054/tuld.1998.0002

[40] L. A. Basso and J. S. Blanchard, "Resistance to Antitubercular Drugs," *Advances in Experimental Medicine and Biology*, Vol. 456, 1998, pp. 115-144. doi:10.1007/978-1-4615-4897-3_7

[41] R. Johnson, *et al.*, "Drug Resistance in *Mycobacterium tuberculosis*," *Current Issues in Molecular Biology*, Vol. 8, No. 2, 2006, pp. 97-111.

[42] A. Banerjee, *et al.*, "*inhA*, a Gene Encoding a Target for Isoniazid and Ethionamide in *Mycobacterium tuberculosis*," *Science*, Vol. 263, No. 5144, 1994, pp. 227-230. doi:10.1126/science.8284673

[43] A. Dessen, *et al.*, "Crystal Structure and Function of the Isoniazid Target of *Mycobacterium tuberculosis*," *Science*, Vol. 267, No. 5204, 1995, pp. 1638-1641. doi:10.1126/science.7886450

[44] K. Mdluli, *et al.*, "Inhibition of a *Mycobacterium tuberculosis* Beta-Ketoacyl ACP Synthase by Isoniazid," *Science*, Vol. 280, No. 5369, 1998, pp. 1607-1610. doi:10.1126/science.280.5369.1607

[45] R. A. Slayden and C. E. Barry, "The Role of KasA and KasB in the Biosynthesis of Meromycolic Acids and Isoniazid Resistance in *Mycobacterium tuberculosis*," *Tuberculosis* (*Edinb*), Vol. 82, No. 4-5, 2002, pp. 149-160. doi:10.1054/tube.2002.0333

[46] A. S. Lee, *et al.*, "Contribution of KasA Analysis to Detection of Isoniazid-Resistant *Mycobacterium tuberculosis* in Singapore," *Antimicrobial Agents and Chemotherapy*, Vol. 43, No. 8, 1999, pp. 2087-2089.

[47] A S. Piatek, *et al.*, "Genotypic Analysis of *Mycobacterium tuberculosis* in Two Distinct Populations Using Molecular Beacons: Implications for Rapid Susceptibility Testing," *Antimicrobial Agents and Chemotherapy*, Vol. 44, No. 1, 2000, pp. 103-110. doi:10.1128/AAC.44.1.103-110.2000

[48] A. S. Lee, A. S. Teo and S. Y. Wong, "Novel Mutations in *ndh* in Isoniazid-Resistant *Mycobacterium tuberculosis* Isolates," *Antimicrobial Agents and Chemotherapy*, Vol. 45, No. 7, 2001, pp. 2157-2159. doi:10.1128/AAC.45.7.2157-2159.2001

[49] C. Vilcheze, *et al.*, "Altered NADH/NAD⁺ Ratio Mediates Coresistance to Isoniazid and Ethionamide in Mycobacteria," *Antimicrobial Agents and Chemotherapy*, Vol. 49, No. 2, 2005, pp. 708-720. doi:10.1128/AAC.49.2.708-720.2005

[50] H. R. van Doorn, *et al.*, "Public Health Impact of Isoniazid-Resistant *Mycobacterium tuberculosis* Strains with a Mutation at Amino-Acid Position 315 of KatG: A Decade of Experience in The Netherlands," *Clinical Microbiology and Infection*, Vol. 12, No. 8, 2006, pp. 769-775.

[51] M. S. Silva, *et al.*, "Mutations in *katG*, *inhA*, and *ahpC* Genes of Brazilian Isoniazid-Resistant Isolates of *MycoBacterium tuberculosis*," *Journal of Clinical Microbiology*, Vol. 41, No. 9, 2003, pp. 4471-4474. doi:10.1128/JCM.41.9.4471-4474.2003

[52] S. Ahmad and E. "Mokaddas, Contribution of AGC to ACC and Other Mutations at Codon 315 of the *katG* Gene in Isoniazid-Resistant *Mycobacterium tuberculosis* Isolates from the Middle East," *International Journal of Antimicrobial Agents*, Vol. 23, No. 5, 2004, pp. 473-479. doi:10.1016/j.ijantimicag.2003.10.004

[53] S. Borukhov and E. Nudler, "RNA Polymerase Holoenzyme: Structure, Function and Biological Implications," *Current Opinion in Microbiologyl*, Vol. 6, No. 2, 2003, pp. 93-100. doi:10.1016/S1369-5274(03)00036-5

[54] D. J. Jin and C. A. Gross, "Three *rpoBC* Mutations that Suppress the Termination Defects of *rho* Mutants also Affect the Functions of *nusA* Mutants," *Molecular and General Genetics*, Vol. 216, No. 2-3, 1989, pp. 269-275. doi:10.1007/BF00334365

[55] L. Minakhin, *et al.*, "Bacterial RNA Polymerase Subunit

Omega and Eukaryotic RNA Polymerase Subunit RPB6 are Sequence, Structural, and Functional Homologs and Promote RNA Polymerase Assembly," *Proceeding of the National Academy of Sciences of the Unite States of Aaerica*, Vol. 98, No. 3, 2001, pp. 892-897.

[56] Global and A. TB, "Rifampin," *Tuberculosis* (*Edinb*), Vol. 88, No. 2, 2008, pp. 151-154. doi:10.1016/S1472-9792(08)70024-6

[57] D. A. Mitchison, "The Action of Antituberculosis Drugs in Short-Course Chemotherapy," *Tubercle*, Vol. 66, No. 3, 198, pp. 219-225.

[58] H. Traore, *et al.*, "Detection of Rifampicin Resistance in *Mycobacterium tuberculosis* Isolates from Diverse Countries by a Commercial Line Probe Assay as an Initial Indicator of Multidrug Resistance," *The International Journal of Tuberculosis and Lung Disease*, Vol. 4, No. 5, 2000, pp. 481-484.

[59] A. Telenti, *et al.*, "Direct, Automated Detection of Rifampin-Resistant *Mycobacterium tuberculosis* by Polymerase Chain Reaction and Single-Strand Conformation Polymorphism Analysis," *Antimicrobial Agents and Chemotherapy*, Vol. 37, No. 10, 1993, pp. 2054-2058.

[60] G. Pozzi, *et al.*, "*rpoB* Mutations in Multidrug-Resistant Strains of *Mycobacterium tuberculosis* Isolated in Italy," *Journal of Clinical Microbiology*, Vol. 37, No. 4, 1999, pp. 1197-1199.

[61] P. Matsiota-Bernard, G. Vrioni and E. Marinis, "Characterization of *rpoB* Mutations in Rifampin-Resistant Clinical *Mycobacterium tuberculosis* Isolates from Greece," *Journal of Clinical Microbiology*, Vol. 36, No. 1, 1998, pp. 20-23.

[62] A. R. Valim, *et al.*, "Mutations in the *rpoB* Gene of Multidrug-Resistant *Mycobacterium tuberculosis* Isolates from Brazil," *Journal of Clinical Microbiology*, Vol. 38, No. 8, 2000, pp. 3119-3122.

[63] L. K. Yuen, D. Leslie and P. J. Coloe, "Bacteriological and Molecular Analysis of Rifampin-Resistant *Mycobacterium tuberculosis* Strains Isolated in Australia," *Journal of Clinical Microbiology*, Vol. 37, No. 12, 1999, pp. 3844-3850.

[64] M. Heep, *et al.*, "Frequency of *rpoB* Mutations Inside and Outside the Cluster I Region in Rifampin-Resistant Clinical *Mycobacterium tuberculosis* Isolates," *Journal of Clinical Microbiology*, Vol. 39, No. 1, 2001, pp. 107-110. doi:10.1128/JCM.39.1.107-110.2001

[65] D. Hillemann, *et al.*, "Use of the Genotype MTBDR Assay for Rapid Detection of Rifampin and Isoniazid Resistance in *Mycobacterium tuberculosis* Complex Isolates," *Journal of Clinical Microbiology*, Vol. 43, No. 8, 2005, pp. 3699-3703. doi:10.1128/JCM.43.8.3699-3703.2005

[66] L. Herrera, *et al.*, "Molecular Analysis of Rifampicin-Resistant *Mycobacterium tuberculosis* Isolated in Spain (1996-2001). Description of New Mutations in the *rpoB* Gene and Review of the Literature," *International Journal of Antimicrobial Agents*, Vol. 21, No. 5, 2003, pp. 403-408. doi:10.1016/S0924-8579(03)00036-0

[67] A. Telenti, *et al.*, "Detection of Rifampicin-Resistance Mutations in *Mycobacterium tuberculosis*," *The Lancet*, Vol. 341, No. 8846, 199, pp. 647-650.

[68] D. L. Williams, *et al.*, "Contribution of *rpoB* Mutations to Development of Rifamycin Cross-Resistance in *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 42, No. 7, 1998, pp. 1853-1857.

[69] N. Siddiqi, *et al.*, "Molecular Characterization of Multidrug-Resistant Isolates of *Mycobacterium tuberculosis* from Patients in North India," *Antimicrobial Agents and Chemotherapy*, Vol. 46, No. 2, 2002, pp. 443-450. doi:10.1128/AAC.46.2.443-450.2002

[70] V. Kapur, *et al.*, "Rapid Mycobacterium Species Assignment and Unambiguous Identification of Mutations Associated with Antimicrobial Resistance in *Mycobacterium tuberculosis* by Automated DNA Sequencing," *Archives of Pathology & Laboratory Medicine*, Vol. 119, No. 2, 1995, pp. 131-138.

[71] S. Morris, *et al.*, "Molecular Mechanisms of Multiple Drug Resistance in Clinical Isolates of *Mycobacterium tuberculosis*," *Journal of Infectious Diseases*, Vol. 171, No. 4, 1995, pp. 954-960. doi:10.1093/infdis/171.4.954

[72] S. Ahmad, *et al.*, "Characterization of *rpoB* Mutations in Rifampin-Resistant *Mycobacterium tuberculosis* Isolates from the Middle East," *Diagnostic Microbiology and Infectious Disease*, Vol. 38, No. 4, 2000, pp. 227-232. doi:10.1016/S0732-8893(00)00200-5

[73] V. Kapur, *et al.*, "Characterization by Automated DNA Sequencing of Mutations in the Gene (*rpoB*) Encoding the RNA Polymerase Beta Subunit in Rifampin-Resistant *Mycobacterium tuberculosis* Strains from New York City and Texas," *Journal of Clinical Microbiology*, Vol. 32, No. 4, 1994, pp. 1095-1098.

[74] H. Taniguchi, *et al.*, "Rifampicin Resistance and Mutation of the *rpoB* Gene in *Mycobacterium tuberculosis*," *FEMS Microbiology Letters*, Vol. 144, No. 1, 1996, pp. 103-108. doi:10.1111/j.1574-6968.1996.tb08515.x

[75] S. Spindola de Miranda, *et al.*, "Mutations in the *rpoB* Gene of Rifampicin-Resistant *Mycobacterium tuberculosis* Strains Isolated in Brazil and France," *Memórias do Instituto Oswaldo Cruz*, Vol. 96, No. 2, 2001, pp. 247-250. doi:10.1590/S0074-02762001000200019

[76] Y. Zhang, *et al.*, "Mode of Action of Pyrazinamide: Disruption of *Mycobacterium tuberculosis* Membrane Transport and Energetics by Pyrazinoic Acid," *Journal Antimicrobial Chemotheapyr*, Vol. 52, No. 5, 2003, pp. 790-795. doi:10.1093/jac/dkg446

[77] M. M. Wade and Y. Zhang, "Anaerobic Incubation Conditions Enhance Pyrazinamide Activity Against *Mycobacterium tuberculosis*," *Journal of Medical Microbiology*, Vol. 53, Part 8, 2004, pp. 769-773. doi:10.1099/jmm.0.45639-0

[78] M. M. Wade and Y. Zhang, "Effects of Weak Acids, UV and Proton Motive Force Inhibitors on Pyrazinamide Activity Against *Mycobacterium tuberculosis in Vitro*," *Journal Antimicrobial Chemotheapyr*, Vol. 58, No. 5, 2006, pp. 936-941. doi:10.1093/jac/dkl358

[79] A. Scorpio and Y. Zhang, "Mutations in *pncA*, a Gene Encoding Pyrazinamidase/Nicotinamidase, Cause Resistance to the Antituberculous Drug Pyrazinamide in Tubercle Bacillus," *Nature Medicine*, Vol. 2, No. 6, 1996, pp. 662-667. doi:10.1038/nm0696-662

[80] P. Sheen, *et al.*, "Effect of Pyrazinamidase Activity on Pyrazinamide Resistance in *Mycobacterium tuberculosis*," *Tuberculosis* (*Edinb*), Vol. 89, No. 2, 2009, pp. 109-113. doi:10.1016/j.tube.2009.01.004

[81] Y. Zhang, *et al.*, "Role of Acid pH and Deficient Efflux of Pyrazinoic Acid in Unique Susceptibility of *Mycobacterium tuberculosis* to Pyrazinamide," *Journal of Bacteriology*, Vol. 181, No. 7, 1999, pp. 2044-2049.

[82] Global and A. TB, "Pyrazinamide," *Tuberculosis* (*Edinb*), Vol. 82, No. 2, 2008, pp. 141-144.

[83] W. R. Butler and J. O. Kilburn, "Susceptibility of *Mycobacterium tuberculosis* to Pyrazinamide and Its Relationship to Pyrazinamidase Activity," *Antimicrobial Agents and Chemotherapy*, Vol. 24, No. 4, 1983, pp. 600-601.

[84] J. Sekiguchi, *et al.*, "Detection of Multidrug Resistance in *Mycobacterium tuberculosis*," *Journal of Clinical Microbiology*, Vol. 45, No. 1, 2007, pp. 179-192. doi:10.1128/JCM.00750-06

[85] J. K. McClatchy, A. Y. Tsang and M. S. Cernich, "Use of Pyrazinamidase Activity on *Mycobacterium tuberculosis* as a Rapid Method for Determination of Pyrazinamide Susceptibility," *Antimicrobial Agents and Chemotherapy*, Vol. 20, No. 4, 198, pp. 556-557.

[86] A. Scorpio, *et al.*, "Characterization of *pncA* Mutations in Pyrazinamide-Resistant *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 41, No. 3, 1997, pp. 540-543.

[87] N. Lemaitre, *et al.*, "Characterization of New Mutations in Pyrazinamide-Resistant Strains of *Mycobacterium tuberculosis* and Identification of Conserved Regions Important for the Catalytic Activity of the Pyrazinamidase pncA," *Antimicrobial Agents and Chemotherapy*, Vol. 43, No. 7, 1999, pp. 1761-1763.

[88] F. Rodrigues Vde, *et al.*, "Characterization of *pncA* Mutations in Pyrazinamide-Resistant *Mycobacterium tuberculosis* in Brazil," *Antimicrobial Agents and Chemotherapy*, Vol. 49, No. 1, 2005, pp. 444-446. doi:10.1128/AAC.49.1.444-446.2005

[89] K. Takayama and J. O. Kilburn, "Inhibition of synthesis of arabinogalactan by ethambutol in *Mycobacterium smegmatis*," *Antimicrobial Agents and Chemotherapy*, Vol. 33, No. 9, 1989, pp. 1493-1499.

[90] S. Sreevatsan, *et al.*, "Ethambutol Resistance in *Mycobacterium tuberculosis*: Critical Role of *embB* Mutations," *Antimicrobial Agents and Chemotherapy*, Vol. 41, No. 8, 1997, pp. 1677-1681.

[91] A. E. Belanger, *et al.*, "The *embAB* Genes of Mycobacterium Avium Encode an Arabinosyl Transferase Involved in Cell Wall Arabinan Biosynthesis that is the Target for the Antimycobacterial Drug Ethambutol," *Proceeding of the National Academy of the Sciences of the United States of America*, Vol. 93, No. 21, 1996, pp. 11919-11924.

[92] A. Telenti, *et al.*, "The *emb* Operon, a Gene Cluster of *Mycobacterium tuberculosis* Involved in Resistance to Ethambutol," *Nature Medicine*, Vol. 3, No. 5, 1997, pp. 567-570. doi:10.1038/nm0597-567

[93] A. Jain, *et al.*, "Novel Mutations in *emb B* Gene of Ethambutol Resistant Isolates of *Mycobacterium tuberculosis*: A Preliminary Report," *Indian Journal of Medical Research*, Vol. 128, No. 5, 2008, pp. 634-639.

[94] Global and A. TB, "Ethambutol," *Tuberculosis* (*Edinb*), Vol. 88, No. 2, 2008, pp. 102-105. doi:10.1016/S1472-9792(08)70008-8

[95] S. V. Ramaswamy, *et al.*, "Molecular Genetic Analysis of Nucleotide Polymorphisms Associated with Ethambutol Resistance in Human Isolates of *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 44, No. 2, 2000, pp. 326-336. doi:10.1128/AAC.44.2.326-336.2000

[96] F. Alcaide, G. E. Pfyffer and A. Telenti, "Role of *embB* in Natural and Acquired Resistance to Ethambutol in Mycobacteria," *Antimicrobial Agents and Chemotherapy*, Vol. 41, No. 10, 1997, pp. 2270-2273.

[97] S. Abbadi, *et al.*, "Characterization of IS6110 Restriction Fragment Length Polymorphism Patterns and Mechanisms of Antimicrobial Resistance for Multidrug-Resistant Isolates of *Mycobacterium tuberculosis* from a Major Reference Hospital in Assiut, Egypt," *Journal of Clinical Microbiology*, Vol. 39, No. 6, 2001, pp. 2330-2334. doi:10.1128/JCM.39.6.2330-2334.2001

[98] J. Davies, L. Gorini and B. D. Davis, "Misreading of RNA Codewords Induced by Aminoglycoside Antibiotics," *Molecular Pharmacology*, Vol. 1, No. 1, 1965, pp. 93-106.

[99] Heifets, L. D., E., "Clinical Mycobateriology Laboratory. Tuberculosis and the Tubercle Bacillus," In: S. E. Cole, K. McMurray and D. Jacobs, Eds., *Tuberculosis and the Tubercle Bacillus*, Washington DC, 2005, pp. 61-62.

[100] Global and A. TB, "Streptomycin," *Tuberculosis* (*Edinb*), Vol. 82, No. 2, 2008, pp. 162-163.

[101] M. Finken, *et al.*, "Molecular Basis of Streptomycin Resistance in *Mycobacterium tuberculosis*: Alterations of the Ribosomal Protein *S*12 Gene and Point Mutations within a Functional 16S Ribosomal RNA Pseudoknot," *Molecular Microbiology*, Vol. 9, No. 6, 1993, pp. 1239-1246. doi:10.1111/j.1365-2958.1993.tb01253.x

[102] J. Nair, *et al.*, "The *rpsL* Gene and Streptomycin Resistance in Single and Multiple Drug-Resistant Strains of *Mycobacterium tuberculosis*," *Molecular Microbiology*, Vol. 10, No. 3, 1993, pp. 521-527. doi:10.1111/j.1365-2958.1993.tb00924.x

[103] A. P. Carter, *et al.*, "Functional Insights from the Structure of the 30S Ribosomal Subunit and Its Interactions with Antibiotics," *Nature*, Vol. 407, No. 6802, 2000, pp. 340-348. doi:10.1038/35030019

[104] A. Meier, *et al.*, "Correlation of Molecular Resistance Mechanisms and Phenotypic Resistance Levels in Streptomycin-Resistant *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 40, No. 11, 1996, pp. 2452-2454.

[105] CDC, "Emergence of *Mycobacterium tuberculosis* with Extensive Resistance to Second-Line Drugs—Worldwide, 2000-2004," *Morbidity Mortality Weekly Report*, Vol. 55, No. 11, 2006, pp. 301-305.

[106] T. H. Holtz and J. P. "Cegielski, Origin of the Term XDR-TB," *European Respiratory Journal*, Vol. 30, No. 2,

2007, p. 396. doi:10.1183/09031936.00042607

[107] K. Drlica and M. Malik, "Fluoroquinolones: Action and Resistance," *Current Topics in Medicinal Chemistry*, Vol. 3, No. 3, 2003, pp. 249-282. doi:10.2174/1568026033452537

[108] H. E. Takiff, *et al.*, "Cloning and Nucleotide Sequence of *Mycobacterium tuberculosis gyrA* and *gyrB* Genes and Detection of Quinolone Resistance Mutations," *Antimicrobial Agents and Chemotherapy*, Vol. 38, No. 4, 1994, pp. 773-780.

[109] J. Y. Wang, *et al.*, "Fluoroquinolone Resistance in *Mycobacterium tuberculosis* Isolates: Associated Genetic Mutations and Relationship to Antimicrobial Exposure," *Journal of Antimicrobial Chemotherapy*, Vol. 59, No. 5, 2007, pp. 860-865. doi:10.1093/jac/dkm061

[110] A. S. Lee, *et al.*, "Characterization of Pyrazinamide and Ofloxacin Resistance among Drug Resistant *Mycobacterium tuberculosis* Isolates from Singapore," *International Journal of Infectious Diseases*, Vol. 6, No. 1, 2002, pp. 48-51. doi:10.1016/S1201-9712(02)90136-0

[111] A. F. Cheng, *et al.*, "Multiplex PCR Amplimer Conformation Analysis for Rapid Detection of *gyrA* Mutations in Fluoroquinolone-Resistant *Mycobacterium tuberculosis* Clinical Isolates," *Antimicrobial Agents and Chemotherapy*, Vol. 48, No. 2, 2004, pp. 596-601. doi:10.1128/AAC.48.2.596-601.2004

[112] O. V. Antonova, *et al.*, "Detection of Mutations in *Mycobacterium tuberculosis* Genome Determining Resistance to Fluoroquinolones by Hybridization on Biological Microchips," *Bulletin of Experimental Biology and Medicine*, Vol. 145, No. 1, 2008, pp. 108-113. doi:10.1007/s10517-008-0034-5

[113] S. S. Hegde, *et al.*, "A Fluoroquinolone Resistance Protein from *Mycobacterium tuberculosis* That Mimics DNA," *Science*, Vol. 308, No. 5727, 2005, pp. 1480-1483. doi:10.1126/science.1110699

[114] G .J. Alangaden, *et al.*, "Mechanism of Resistance to Amikacin and Kanamycin in *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 42, No. 5, 1998, pp. 1295-1297.

[115] Y. Suzuki, *et al.*, "Detection of Kanamycin-Resistant *Mycobacterium tuberculosis* by Identifying Mutations in the 16S rRNA Gene," *Journal of Clinical Microbiology*, Vol. 36, No. 5, 1998, pp. 1220-1225.

[116] "Amikacin," *Tuberculosis* (*Edinb*), Vol. 88, No. 2, 2008, pp. 87-88. doi:10.1016/S1472-9792(08)70003-9

[117] Global and A. TB, "Capreomycin," *Tuberculosis* (*Edinb*), Vol. 88, No. 2, 200, pp. 89-91.

[118] C. E. Maus, B. B. Plikaytis and T. M. Shinnick, "Mutation of *tlyA* Confers Capreomycin Resistance in *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 49, No. 2, 2005, pp. 571-577. doi:10.1128/AAC.49.2.571-577.2005

[119] S. K. Johansen, *et al.*, "Capreomycin Binds across the Ribosomal Subunit Interface Using tlyA-Encoded 2'-O-Methylations in 16S and 23S rRNAs," *Molecular Cell*, Vol. 32, No. 2, 2006, pp. 173-182. doi:10.1016/j.molcel.2006.05.044

[120] C. E. Maus, B. B. Plikaytis and T. M. Shinnick, "Molecular Analysis of Cross-Resistance to Capreomycin, Kanamycin, Amikacin, and Viomycin in *Mycobacterium tuberculosis*," *Antimicrobial Agents and Chemotherapy*, Vol. 49, No. 8, 2005, pp. 3192-3197. doi:10.1128/AAC.49.8.3192-3197.2005

[121] H. Taniguchi, *et al.*, "Molecular Analysis of Kanamycin and Viomycin Resistance in Mycobacterium Smegmatis by Use of the Conjugation System," *Journal of Bacteriology*, Vol. 179, No. 15, 1997, pp. 4795-4801.

[122] A. R. Baulard, *et al.*, "Activation of the Pro-Drug Ethionamide is Regulated in Mycobacteria," *Journal of Biological Chemistry*, Vol. 275, No. 36, 2000, pp. 28326-28331.

[123] Global and A. TB, "Ethionamide," *Tuberculosis* (*Edinb*), Vol. 88, No. 2, 2008, pp. 106-108. doi:10.1016/S1472-9792(08)70009-X

[124] J. Engohang-Ndong, *et al.*, "EthR, a Repressor of the TetR/CamR Family Implicated in Ethionamide Resistance in Mycobacteria, Octamerizes Cooperatively on its Operator," *Molecular Microbiology*, Vol. 51, No. 1, 2004, pp. 175-188. doi:10.1046/j.1365-2958.2003.03809.x

[125] G. P. Morlock, *et al.*, "*ethA*, *inhA*, and *katG* Loci of Ethionamide-Resistant Clinical *Mycobacterium tuberculosis* Isolates," *Antimicrobial Agents and Chemotherapy*, Vol. 47, No. 12, 2003, pp. 3799-3805. doi:10.1128/AAC.47.12.3799-3805.2003

[126] G. Di Perri and S. Bonora, "Which Agents should We Use for the Treatment of Multidrug-Resistant *Mycobacterium tuberculosis*?" *Journal of Antimicrobial Chemotherapy*, Vol. 54, No. 3, 2004, pp. 593-602. doi:10.1093/jac/dkh377

[127] T. Cohen, *et al.*, "Mathematical Models of the Epidemiology and Control of Drug-Resistant TB," *Expert Review of Respiratory Medicine*, Vol. 3, No. 1, 2009, pp. 67-79. doi:10.1586/17476348.3.1.67

[128] M. Barnard, *et al.*, "Rapid Molecular Screening for Multidrug-Resistant Tuberculosis in a High-Volume Public Health Laboratory in South Africa," *American Journal of Respiratory and Critical Care Medicine*, Vol. 177, No. 7, 2008, pp. 787-792. doi:10.1164/rccm.200709-1436OC

[129] L. Caviedes, *et al.*, "Rapid, Efficient Detection and Drug Susceptibility Testing of *Mycobacterium tuberculosis* in Sputum by Microscopic Observation of Broth Cultures," *Journal of Clinical Microbiology*, Vol. 38, No. 3, 2000, pp. 1203-1208.

[130] S. Y. Lin, *et al.*, "Rapid Detection of Isoniazid and Rifampin Resistance Mutations in *Mycobacterium tuberculosis* Complex from Cultures or Smear-Positive Sputa by Use of Molecular Beacons," *Journal of Clinical Microbiology*, Vol. 42, No. 9, 2004, pp. 4204-4208. doi:10.1128/JCM.42.9.4204-4208.2004

[131] A. Hamid Salim, *et al.*, "Early and Rapid Microscopy-Based Diagnosis of True Treatment Failure and MDR-TB," *International Journal of Tuberculosis and Lung Disease*, Vol. 10, No. 11, 2006, pp. 1248-1254.

[132] M. Pai, *et al.*, "Bacteriophage-Based Assays for the Rapid Detection of Rifampicin Resistance in *Mycobacterium tuberculosis*: A Meta-Analysis," *Journal of Infection*, Vol.

51, No. 3, 2005, pp. 175-187.
doi:10.1016/j.jinf.2005.05.017

[133] A. Martin, F. Portaels and J. C. Palomino, "Colorimetric Redox-Indicator Methods for the Rapid Detection of Multidrug Resistance in *Mycobacterium tuberculosis*: A Systematic Review and Meta-Analysis," *Journal of Antimicrobial Chemotherapy*, Vol. 59, No. 2, 2007, pp. 175-183. doi:10.1093/jac/dkl477

[134] A. Martin, *et al.*, "Nitrate Reductase Assay for the Rapid Detection of Pyrazinamide Resistance in *Mycobacterium tuberculosis* Using Nicotinamide," *Journal of Antimicrobial Chemotherapy*, Vol. 61, No. 1, 2008, pp. 123-127. doi:10.1093/jac/dkm418

[135] C. Y. Chiang, R. Centis and G. B. Migliori, "Drug-Resistant Tuberculosis: Past, Present, Future," *Respirology*, Vol. 15, No. 3, 2010, pp. 413-432. doi:10.1111/j.1440-1843.2010.01738.x