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Review Article

Therapeutic Activities of Anti-Tuberculosis (TB) Drugs and Molecular Resistance Mechanisms of Multidrug and Extensively Drug-Resistant-TB

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Abstract

Tuberculosis (TB) is a major global health problem. It is estimated that more than 2 billion people are latently infected with *Mycobacterium tuberculosis* (*M. tuberculosis*), resulting in approximately 3 million deaths worldwide per year. Among the unique features of *M. tuberculosis* is its ability to establish persistent infection, requiring prolonged antibiotic treatment in order to achieve clinical cure. The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Control of TB is hampered by the emergence of multidrug resistance (MDR), defined as resistance to at least rifampicin and isoniazid, two key drugs in the treatment of the disease. More recently, severe forms of drug resistance such as extensively drug-resistant (XDR) TB have been emerged. After the discovery of several drugs with anti-TB activity, multidrug therapy became fundamental for control of the disease. Drug resistance in *M. tuberculosis* arises from spontaneous chromosomal mutations at low frequency. Clinical drug-resistant TB largely occurs as a result of man-made selection during disease treatment of these genetic alterations through erratic drug supply, suboptimal physician prescription and poor patient adherence. Better knowledge of the mechanisms of drug resistance in TB and the molecular mechanisms involved will help us to improve current techniques for rapid detection and will also stimulate the exploration of new targets for drug activity and drug development. This paper presents an updated review of the mechanisms and molecular basis of drug resistance in *M. tuberculosis* and therapeutic activities of anti- TB drugs. It also fills our gaps in the current knowledge of the molecular mechanisms of drug resistance to the main old and new anti- TB drugs.

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Introduction

Tuberculosis (TB) is one of the well-known bacterial infectious diseases caused by the obligate human pathogen known as *Mycobacterium tuberculosis*. *M. tuberculosis* and seven very closely related mycobacterial species such as *M. bovis, M. africanum, M. microti, M. caprae, M. pinnipedii, M. canetti* and *M. mungi* are together known as the *M. tuberculosis* complex (Birhanu and Fisseha, 2013). Historically, TB

has been associated with significant morbidity and mortality, and still remains a major global health problem. It is estimated that 2 billion people are latently infected with *M. tuberculosis*, resulting in approximately 3 million deaths worldwide per year (Anastasia and Petros, 2012). In 2010, it was estimated that there were 8.8 million incident cases of TB, 1.1 million deaths from TB among HIV-negative people and an additional 0.35 million deaths from HIV-associated TB (Jansy et al., 2012). Currently, (TB) is a medical, social, and economic disaster of huge magnitude in the world (Birhanu and Fisseha, 2013). Among the unique features of this organism is its ability to establish persistent infection and requiring prolonged antibiotic treatment in order to achieve clinical cure (Anastasia and Petros, 2012).

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents (Fred, 2006; Michael and Stuart, 2007). Multidrug-resistant pathogens historically were limited to the hospital setting. In the 1990s, multidrug-resistant pathogens were described to be affecting outpatients in health care–associated settings (nursing homes, dialysis centers, infusion centers, among patients recently hospitalized). More recently, multidrug-resistant pathogens have become major issues in the community, affecting persons with limited or in many cases no contact with health care (Chen et al., 2011).

Some of the most problematic MDR organisms that are encountered currently include *Pseudomonas aeruginosa, Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella pneumoniae* bearing extended-spectrum β-lactamases (ESBL), vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA) *Staphylococcus aureus* , *Salmonella* spp. and M/XDR *M. tuberculosis* (Michael and Stuart, 2007; Shamebo et al., 2016).

The history of TB changed dramatically after the introduction of the first drugs with anti-mycobacterial activity. However, not long after the first antibiotic was introduced in 1944, drug resistance emerged, mainly due to the use of streptomycin as mono therapy. With the discovery of several other drugs with anti-TB activity, multidrug therapy became fundamental for the control of the disease by promoting the cure of the patients and interrupting the chain of transmission (Pedro and Juan, 2011).

Inadequate drug treatment of an individual with TB will kill the majority of their bacteria but will permit the growth of the small number of resistant organisms within that bacterial population which are arising by spontaneous mutation. A population wholly resistant to a single drug then emerges, and continuing inadequate treatment goes on to select from among this population the small number of organisms which have mutated to have further drug resistance. Resistance to one drug may therefore become resistance to two drugs, and then sequentially too many drugs. Any TB resistant to at least isoniazid and rifampicin is considered as MDR (Faustini et al., 2006).

According to WHO/HTM/TB (2010) explanation, XDR-TB is a form of TB caused by bacteria that are resistant to isoniazid and rifampicin (i.e. MDR-TB) as well as any fluoroquinolone and any of the second-line anti-TB injectable drugs (amikacin, kanamycin or capreomycin). These forms of TB do not respond to the standard six month treatment with first-line anti-TB drugs and can take up to two years or more to treat with drugs that are less potent, more toxic and much more expensive (Pedro and Juan, 2011). M/XDR-TB results from either primary infection with resistant bacteria or may develop in the course of a patient's treatment (WHO/HTM/TB/2010; Zhang and Yew, 2009). M/XDR-TB cases may thus arise by direct transmission of an M/XDR strain from one individual to another, but also by inadequate treatment of an individual who was initially infected by a fully sensitive strain (Faustini et al., 2006).

Either transmission of M/XDR strains or selection of single drug resistant strains may have contributed to the increase in the prevalence of M/XDR-TB. The prevalence of infection among contacts of MDR-TB cases is similar to the prevalence among contacts of cases without MDR-TB. In closed communities such as prisons and hospitals, MDR-TB has been transmitted between immune competent as well as immune deficient individuals (Faustini et al., 2006). According to WHO/HTM/TB (2010) report in 2008, an estimated 390 000–510 000 cases of MDR-TB emerged globally. The main distribution of tuberculosis is located in many Asian and African countries (Faustini et al., 2006). Among all incident TB cases globally, 3.6% are estimated to have MDR-TB. Almost 50% of MDR-TB cases worldwide are estimated to occur in China and India. In 2008, MDR-TB caused an estimated 150 000 deaths (WHO/HTM/TB/2010).

Genetic aspects in the development of drug- resistant tuberculosis

Mutations in the genome of *M. tuberculosis* that can confer resistance to anti-TB drugs occur spontaneously with an estimated frequency of 3.5×10^{-6} for isoniazid, and 3.1×10^{-8} for rifampicin (Johnson et al., 2009). Because mutations resulting in drug resistance are unlinked, the probability of developing bacillary resistance to three drugs used simultaneously becomes 10^{-18} to 10^{-20}. In theory, the chance of drug resistance is thus virtually non-existent when three effective drugs are used in combination for TB treatment (Zhang and Yew, 2009)**.** Amplification of the afore-mentioned genetic mutation through human error results in clinically drug-resistant TB. These include 'mono therapy' due to irregular drug supply, inappropriate doctor prescription and, most importantly, poor patient adherence to treatment. Subsequent transmission of resistant *M. tuberculosis* strains from the index patient to others aggravates the problem (Zhang and Yew, 2009).

Mobile genetic elements such as plasmids and transposons, which are known to mediate drug resistance in various bacterial species, do not do so in *M. tuberculosis*, but acquired drug resistance is exclusively due to chromosomal alterations such as mutations or deletions. These chromosomal alterations affect either the drug target itself or bacterial enzymes activating/modifying the drug. Drug resistance in *M. tuberculosis* mostly occurs when resistant mutants that are present naturally in the mycobacterial population are selected out by inadequate or interrupted treatment (Zhang and Yew, 2009). In theory, the effective presence of a mutant which is resistant to 2 drugs would require a population of 10^{12}– 10^{16} mycobacterial cells. This concept provides the basis for the successful use of combination drug therapy to prevent the emergence of resistance. Thus, clinical poly- or multidrug resistance in *M. tuberculosis* is not due to a single genetic locus, such as up regulation of an efflux pump or induction of a transcriptional regulator, but rather due to an accumulation of multiple different mutations (Erik, 2011).

The M/XDR phenotype is caused by sequential accumulation of mutations in different genes (Zhang and Yew, 2009). In general, there is a clear correlation between the genetic mechanism and the resistance phenotype. Thus, mutations in *rpsL* (Streptomycin), *rpoB* (Rifampin) or 16S ribosomal RNA (KAN, AMK; 2- deoxystreptamine aminoglycosides) are associated with high-level drug resistance, and mutations in *gldB* (Streptomycin), *eis* (kanamycin), and *inhA* (Isoniazid) confer a low- level resistance phenotype. Resistance-conferring chromosomal alterations in a drug target gene in a clinical isolates are highly restricted. Presumably this reflects the *in vivo* selection for resistance mutations which maintain gene function, readily explaining the predominance of certain resistance mutations. In contrast, resistance-conferring chromosomal alterations in genes involved in pro-drug conversion, for example *pncA* and *ethA*, often display a wide diversity, indicating that there is little functional constraint as a loss of gene function phenotype is apparently well tolerated (Erik, 2011).

According to Erik (2011) report, nucleic acid sequence polymorphisms and unknown genetic alterations would be expected to affect the phenotype of a chromosomal resistance determinant. Significant levels of phenotypic heterogeneity for a given resistance mutation have been observed only rarely, for example the *katG S315T* alteration and (Isoniazid) resistance. Also, resistance to a single drug may involve multiple genetic alterations locating to different genes, as well as multiple genetic alterations within a single gene. Presumably, this accumulation of various resistance mutations, all associated with resistance to a single drug, will either affect (increase) phenotypic resistance.

Intrinsic and acquired drug resistance

The development of drug resistance in TB is classified as acquired resistance when drug resistant mutants are selected as a result of ineffective treatment or as primary resistance when a patient is infected with a resistant strain (Johnson et al., 2009).

Intrinsic resistance

Intrinsic resistance refers to the innate ability of a bacterium to resist the activity of a particular antimicrobial agent through its inherent structural or functional characteristics (Anastasia and Petros, 2012). This passive resistance is a consequence of general adaptive processes that are not necessary linked to a given class of antimicrobials. For example the presence of genes affording resistance to self-produced antibiotics, the outer membrane of Gram-negative bacteria, absence of an uptake transport system for the antimicrobial or general absence of the target or reaction

hit by the antimicrobial (Wright,2005; Katrijn and Arthur, 2009).

Intrinsic drug resistance of *M. tuberculosis* has traditionally been attributed to the unusual structure of its mycolic acid-containing cell wall that gives the bacteria a low permeability for many compounds such as antibiotics and other chemotherapeutic agents (Pedro and Juan, 2011). The role of efflux mechanisms has also been recognized as an important factor in the natural resistance of mycobacteria against antibiotics such as tetracycline, fluoroquinolones and aminoglycosides, among others (Rossi, 2006).

In *Mycobacterium* smegmatis, it has been shown that mutants lacking the major porin *mspA* had an increased MIC of the B-lactam antibiotics ampicillin and cefaloridine. Deletion of the *mspA* gene also increased the MIC of vancomycin, erythromycin and rifampicin by 2- to 10-fold (Stephan *et al*., 2004). B-lactam antibiotics bind and inhibit the activities of penicillin-binding proteins involved in cell wall biosynthesis, but *Mycobacteria* possess B-lactamase enzymes that degrade these drugs. In *M. tuberculosis*, B-lactamase activity is encoded by *blaC* and *blaS.* The use of B-lactamase-resistant B-lactam antibiotics or B-lactam in combination with B-lactamase inhibitors has been shown to be effective in killing *M. tuberculosis*. Not only permeability barriers or B-lactamases are responsible for the intrinsic resistance to antibiotics in mycobacteria but also physiological adaptations occurring within the host can also be responsible for antibiotic tolerance (Pedro and Juan, 2011).

Acquired drug resistance

Acquired drug resistance is active and results from changes in the bacterial genome. Resistance in bacteria may be acquired by a mutation and passed vertically by selection to daughter cells. More commonly, resistance is acquired by horizontal transfer of resistance genes between strains and species. Exchange of genes is possible by transformation, transduction or conjugation (Rachakonda, 2004; Katrijn and Arthur, 2009).

Unlike the situation in other bacteria where acquired drug resistance is generally mediated through horizontal transfer by mobile genetic elements, such as plasmids, transposons or integrons, in *M. tuberculosis*, acquired drug resistance is caused mainly by spontaneous mutations in chromosomal genes, producing the selection of resistant strains during sub-optimal drug therapy (Pedro and Juan, 2011). Although no single pleiotropic mutation has been found to cause an MDR phenotype in *M. tuberculosis*, a possible complex association between classical mutations associated with resistance to one drug could be related to initial steps in the resistance to other drugs (Safi, 2008; Pedro and Juan, 2011). It occurs when a microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible. The rate of genetic mutations leading to resistance varies somewhat among anti-tuberculosis drugs, from a frequency of ~10^{-5} -10^{-6} organisms for isoniazid to ~10^{-7}-10^{-8} organisms for rifampin (Karakousis, 2009; Anastasia and Petros, 2012). Fig. 1 shows the main mechanisms of active antimicrobial resistance.

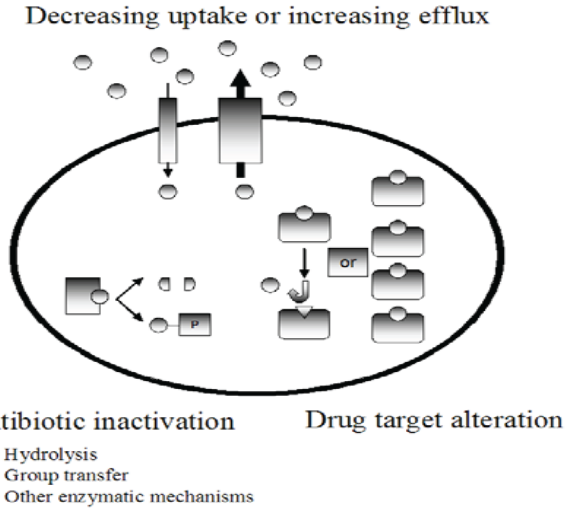

Fig. 1: Main mechanisms of active antimicrobial resistance (Katrijn and Arthur, 2009).

In prokaryotes, spontaneous mutations occur at a low rate of 0.0033 per replication. The mutation rate per base pair is inversely proportional to the genome size. The rate of mutation depends on the nature of the drug selection, but for most of the main anti-TB drugs, this occurs at a rate of 10^{9} mutations per cell division. This is the main reason why anti-TB drugs are given as a combination, as the risk of a mutant containing two resistance mutations is $<10^{18}$ (Pedro and Juan, 2011).

Anti-TB drugs and their molecular mechanisms of action and resistance

Different drugs have many and various ways of action on the antimicrobial agents. Actually used targets for

antimicrobial agents are showmen in the following diagram (Fig. 2).

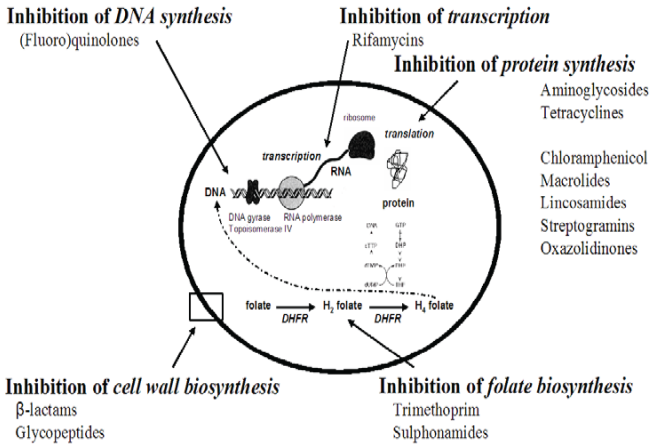

Fig. 2: Actually used targets for antimicrobial agents (Katrijn and Arthur, 2009).

Old TB drugs

Isoniazid (INH)

Isonicotinic acid hydrazide (INH) is a highly specific antimycobacterial agent, being exquisitely potent against the *M. tuberculosis* complex (Erik, 2011). INH is the most widely used first-line anti-tuberculosis drug. INH has been the cornerstone of all effective regimens for the treatment of TB disease and latent infection since its discovery in 1952. *M. tuberculosis* is highly susceptible to INH (minimum inhibitory concentration [MIC] 0.02–0.2 μg/ml), but INH is only active against growing tubercle bacilli, and is not active against non-replicating bacilli or under anaerobic conditions (Zhang and Yew, 2009).

INH has a simple structure containing a pyridine ring and a hydrazide group, with both components being essential for the high activity against *M. tuberculosis* (Pedro and Juan, 2011). Consisting of a pyridine ring and a hydrazide group, INH is a nicotinamide analog, structurally related to the anti-tuberculosis drugs ethionamide and pyrazinamide. Because of its significant bactericidal activity, it has become a critical component of the first-line antituberculous regimens, although in the last two decades resistance to INH has been reported with increasing frequency (Anastasia and Petros, 2012).

Despite this simple structure, the mode of action of isoniazid is more complex and isoniazid-resistant strains had already been isolated as soon as its anti-TB activity was recognized. Very early on, it was also shown that *Mycobacterium bovis* and *M. tuberculosis* isoniazid-resistant strains lacking catalase activity were highly attenuated in guinea pigs (Pedro and Juan, 2011).

INH appears to penetrate host cells readily and diffuses across the *M. tuberculosis* membrane. INH is a pro-drug, requiring oxidative activation by the *M. tuberculosis* catalase-peroxidase enzyme *KatG* (Anastasia and Petros, 2012) to generate a range of highly reactive species which then attack multiple targets in *M. tuberculosis*. The reactive species produced by *KatG*-mediated INH activation include reactive oxygen species such as superoxide, peroxide and hydroxyl radical, (Timmins *et al*., 2004) nitric oxide and reactive organic species such as isonicotinic-acyl radical or anion, and certain electrophilic species (Zhang and Yew, 2009).

Although the active metabolites of INH have been reported to inhibit multiple essential cellular pathways, including synthesis of nucleic acids), phospholipids, and NAD metabolism, the primary pathway responsible for the killing activity of the drug is mycolic acid synthesis. Thus, the activated form of the drug binds tightly to the NADH-dependent enoylacyl carrier protein (ACP) reductase *InhA*, a component of the fatty acid synthase II system of mycobacteria, which is essential for fatty acid elongation (Anastasia and Petros, 2012).

Resistance to INH occurs more frequently than for most anti-tuberculosis drugs, at a frequency of 1 in $10^{5–6}$ bacilli *in vitro*. INH-resistant clinical isolates of *M. tuberculosis* often lose catalase and peroxidase enzyme encoded by *katG*, especially in high-level resistant strains (MIC > 5 μg/ml) (Dalla, 2009). Low-level resistant strains (MIC < 1 μg/ml) often still possess catalase activity (Pedro and Juan, 2011). Mutations in INH-resistant clinical isolates are most commonly detected in the *katG* gene, occurring in 50–80% of cases, thus reducing the ability of the catalase-peroxidase to activate the INH pro-drug. The *katG* gene is located in a highly variable and unstable region of the *M. tuberculosis* genome, with missense and nonsense mutations, insertions, deletions, truncation and, more rarely, full gene deletions observed (Anastasia and Petros, 2012). So far, more than a hundred mutations in *katG* have been reported, with MICs ranging from 0.2 to 256 mg/L (Pedro and Juan, 2011).

Mutation in *kat*G is the main mechanism of INH resistance (Anastasia and Petros, 2012). Resistance to

isoniazid is a complex process. Mutations in several genes, including *katG*, *ahpC*, *inhA*, *kasA* and *ndh*, have all been associated with isoniazid resistance. The most common mutation is S315T, which results in an isoniazid product that is highly deficient in forming the isoniazid-NAD adduct related to isoniazid antimicrobial activity. Activated isoniazid interferes with the synthesis of essential mycolic acids by inhibiting NADH dependent enoyl-ACP reductase, which is encoded by *inhA*. Two molecular mechanisms have been shown to be the main cause for isoniazid resistance: mutations in *katG* and mutations in *inhA*, or more frequently in its promoter region (Pedro and Juan, 2011).

Rifampin

Rifampin (RMP) is a broad-spectrum antibiotic and the most widely used rifamycin to treat TB (Anastasia and Petros, 2012). RMP is an important first-line drug for the treatment of TB. RMP is bactericidal for *M. tuberculosis*, with MICs ranging from 0.05 to 1 μg/ml on solid or liquid media, but the MIC is higher in egg media (MIC = 2.5–10 μg/ml). RMP is active against both growing and stationary phase bacilli with low metabolic activity. The latter activity is related to its high sterilizing activity *in vivo*, correlating with its ability to shorten TB treatment from 12–18 months to 9 months (Zhang and Yew, 2009).

RMP interferes with RNA synthesis by binding to the β subunit of the RNA polymerase (Pedro and Juan, 2011). The RNA polymerase is an oligomer consisting of a core enzyme formed by four chains $\alpha 2\beta\beta'$ in association with the σ subunit to specifically initiate transcription from promoters (Zhang and Yew, 2009). The RMP-binding site is located upstream of the catalytic center and physically blocks the elongation of the RNA. In *M. tuberculosis*, resistance to RMP occurs at a frequency of 10^{-7} to 10^{-8} (Zhang and Yew, 2009).

Mutations in a 'hot-spot' region of 81 base pare of *rpoB* have been found in about 96% of rifampicin-resistant *M. tuberculosis* isolates (Pedro and Juan, 2011; Anastasia and Petros, 2012; Johnson et al., 2009). This region, spanning codons 507–533, is also known as the rifampicin resistance-determining region (RRDR). Mutations in codons 531 and 526 are the most frequently reported mutations in most of the studies (Caws et al., 2006). Some studies have also reported mutations outside of the hot-spot region of *rpoB* in rifampicin-resistant *M. tuberculosis* isolates (Pedro and Juan, 2011; Anastasia and Petros, 2012).

Pyrazinamide (PZA)

Pyrazinamide was discovered in 1952 and introduced into TB chemotherapy in the early 1950s. Its use allowed the length of treatment to be reduced from 9 to 6 months. One key characteristic of pyrazinamide is its ability to inhibit semidormant bacilli residing in acidic environments (Pedro and Juan, 2011). Pyrazinamide is a structural analogue of nicotinamide and is a pro-drug that needs to be converted into its active form, pyrazinoic acid, by the enzyme pyrazinamidase/ nicotinamidase (PZase) (Johnson et al., 2009). PZase is encoded in *M. tuberculosis* by the gene pncA (Pedro and Juan, 2011; Anastasia and Petros, 2012). PZA on the other hand has effective sterilizing activity and shortens the chemotherapeutic regiment from 12 to 6 months. PZA is a pro-drug which is converted to its active form, pyrazinoic acid (POA) by the pyrazinamidase (PZase) encoded by *pncA* (Johnson et al., 2009).

The activity of PZA is highly specific for *M. tuberculosis*, as it has no effect on other mycobacteria (Johnson et al., 2009). PZA enters tubercle bacilli passively and via an ATP-dependent transport system. Intracellular accumulation of the drug occurs because of an inefficient efflux system unique to *M. tuberculosis*. Uptake and intra bacillary accumulation of POA is enhanced when the extracellular pH is acidic. The anti-tuberculosis activity of PZA has been attributed to disruption of the proton motive force required for essential membrane transport functions by POA at acidic pH, although investigation into potential specific cellular targets is ongoing (Anastasia and Petros, 2012).

Mutations in *pncA* are the main mechanisms for pyrazinamide resistance in *M. tuberculosis*. Most alterations occur in a 561 base pair region of the open reading frame or in an 82 base pair region of its putative promoter (Jure et al., 2008). There is a high degree of diversity of *pncA* gene mutations among pyrazinamide-resistant strains; however, some pyrazinamide-resistant strains do not show mutations in *pncA* or its promoter region. In this case, it has been postulated that resistance to pyrazinamide could be due to mutations occurring in an unknown *pncA* regulatory gene (Pedro and Juan, 2011).

An alternative explanation could be the difficulty in performing drug susceptibility testing for pyrazinamide and that these strains are falsely resistant by the phenotypic test. Furthermore, a small proportion of pyrazinamide-resistant strains that have low-level resistance and retain PZase activity are considered to have another alternative mechanism of resistance. The highly specific activity of pyrazinamide for *M. tuberculosis*, with little or no activity against other mycobacteria, can be explained by the fact that *pncA* is altered in many species of mycobacteria; e.g. in *M. bovis* subsp. *bovis*, the natural substitution *H57A* produces a non-effective PZase determining intrinsic resistance to pyrazinamide (Pedro and Juan, 2011). The high specificity of PZA for *M. tuberculosis*, with little or no activity against *M. bovis* and other mycobacteria, is attributable to *pncA* mutations, which render PZase inactive in the latter mycobacterial species (Anastasia and Petros, 2012).

Ethambutol (EMB)

Ethambutol, 2,2′- (1,2-ethanediyldiimino) bis-1-butanol, was first used in 1966 against TB and constitutes, together with isoniazid, rifampicin and pyrazinamide, the first-line drugs currently in use for treatment of the disease (Pedro and Juan, 2011; Anastasia and Petros, 2012; Zhang and Yew, 2009). The MICs of EMB for *M. tuberculosis* are in the range of 0.5–2 μg/ml. EMB is a bacteriostatic agent that is active for growing bacilli and has no effect on non-replicating bacilli (Zhang and Yew, 2009).

Ethambutol is active against multiplying bacilli (Pedro and Juan, 2011). It inhibits 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 (Zhang and Yew, 2009). EMB also has been reported to inhibit several other cellular pathways, including RNA metabolism, transfer of mycolic acids into the cell wall, phospholipid synthesis, and spermidine biosynthesis (Anastasia and Petros, 2012).

Strains resistant to EMB have MICs > 7.5 μg/ml. Mutation to EMB resistance occurs at a frequency of 10^{-5}. Resistance to EMB in *M. tuberculosis* is usually associated with point mutations in the *embCAB* operon (Anastasia and Petros, 2012). Mutations in the *embCAB* operon, in particular *embB*, and occasionally *embC*, are responsible for resistance to EMB. The *emb*B codon 306 mutation is most frequent in clinical isolates resistant to EMB, accounting for as high as 68% resistant strains (Zhang and Yew, 2009).

Genetic and biochemical studies have shown that the *EmbA* and *EmbB* proteins are involved in the formation of the proper terminal hexaarabinofuranoside motif during arabinogalactan synthesis, while *EmbC* is involved in lipoarabinomannan synthesis (Anastasia and Petros, 2012). As the majority of EMB-resistant clinical isolates contain mutations in the *embB* gene, *EmbB* is considered to be the main target of EMB, although X-ray crystallographic data supporting this interaction are lacking. More recently, the most commonly observed mutations in *embB* codon 306 have been reported to be associated with variable degrees of EMB resistance, indicating that such mutations may be necessary but not sufficient for high level EMB resistance (Anastasia and Petros, 2012).

Aminoglycosides (streptomycin, kanamycin/ amikacin/capreomycin)

Streptomycin (SM) is an aminoglycoside antibiotic that is active against a variety of bacterial species, including *M. tuberculosis.* SM kills actively growing tubercle bacilli with MICs of 2–4 μg/ml, (Heifets, 2005) but it is inactive against non-growing or intracellular bacilli (Zhang and Yew, 2009). Aminoglycosides are used currently as second-line drugs primarily in the treatment of MDR-TB (Anastasia and Petros, 2012). SM inhibits protein synthesis by binding to the 30S subunit of bacterial ribosome, causing misreading of the mRNA message during translation. The site of action of SM is the 30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA (Zhang and Yew, 2009).

Resistance to streptomycin and the other aminoglycosides in *M. tuberculosis* usually develops by mutation of the ribosome target binding sites. In *M. tuberculosis* the genetic basis of resistance to streptomycin is mostly due to mutations in *rrs* or *rpsL*, which produce alterations in the streptomycin binding site (Pedro and Juan, 2011). The majority of point mutations resulting in streptomycin resistance occur in *rpsL*, with the most common mutation being *K43R*. Some clinical isolates showing low-level resistance to streptomycin and no mutation in *rpsL* or *rrs* have also been found, and have consequently been theorized as an alternative mechanism for streptomycin resistance. More

recently it has been shown that mutations in *gidB*, which encodes a conserved 7-methylguanosine methyltransferase specific for the 16S rRNA, can confer a low level of streptomycin resistance (Okamoto *et al*., 2007; Spies et al., 2008; Pedro and Juan, 2011).

Fluoroquinolones

The fluoroquinolones (moxifloxacin, gatifloxacin, sparfloxacin, levofloxacin, ofloxacin, and ciprofloxacin), are bactericidal antibiotics with excellent activity against *M. tuberculosis* and are currently used as second-line drugs in TB treatment (Anastasia and Petros, 2012). Currently a new generation of fluoroquinolones, such as moxifloxacin and gatifloxacin, are under clinical evaluation and are being proposed as first-line antibiotics with the goal of shortening the duration of TB treatment (Pedro and Juan, 2011).

In *M. tuberculosis*, only type II topoisomerase (DNA gyrase) is present and thus is the only target for fluoroquinolone activity (Aubry *et al*., 2004). Type II topoisomerase is a tetramer composed of two A and B subunits encoded by the genes *gyrA* and *gyrB*, respectively, that catalyses the super coiling of DNA (Pedro and Juan, 2011). Fluoroquinolone resistance in *M. tuberculosis* is most commonly associated with mutations in the conserved quinolone resistance-determining region (QRDR) of *gyrA* and *gyrB* involved in the interaction between the drug and DNA gyrase (Anastasia and Petros, 2012). The most frequently observed mutations associated with fluoroquinolone resistance in *M. tuberculosis* are at positions Ala-90 and Asp-94 in the *gyrA* gene. Codon 95 contains a naturally occurring polymorphism (*Ser* or *Thr*) that is not related to fluoroquinolone resistance, as it occurs in both fluoroquinolone-susceptible and fluoroquinolone-resistant strains (Anastasia and Petros, 2012; Pedro and Juan, 2011). Interestingly, strains with mutations at position 80 of *gyrA* have been reported to cause hyper susceptibility, especially if present together with other resistance mutations (Aubry et al., 2006; Pedro and Juan, 2011).

Macrolides

Macrolides are broad-spectrum antibiotics (Anastasia and Petros, 2012) which affect the early stage of protein synthesis, namely translocation, by targeting the conserved sequences of the peptidyltransferase centre of the 23S rRNA of the 50S ribosomal subunit (Yoneyama, 2006). This results in a premature detachment of incomplete peptide chains (Katrijn and Arthur, 2009). However, these drugs have limited activities against wild-type *M. tuberculosis* (Anastasia and Petros, 2012). Intrinsic resistance to the macrolides in *M. tuberculosis* has been attributed to low cell wall permeability and expression of the *erm(*37*)* gene encoding a 23S rRNA methyltransferase, which is present in all members of the *M. tuberculosis* complex but absent in nontuberculous mycobacteria (Anastasia and Petros, 2012; Pedro and Juan, 2011).

Ethionamide /prothionamide and thioamides

ETH (2-ethylisonicotinamide) is a derivative of isonicotinic acid, and is bactericidal only against *M. tuberculosis, M. avium-intracellulare* and *M. leprae.* The MICs of ETH for *M. tuberculosis* are 0.5–2 μg/ml in liquid medium, 2.5–10 μg/ml in 7H11 agar, and 5– 20 μg/ml in LJ medium (Zhang and Yew, 2009). Ethionamide (ETH) is an important drug in the treatment of MDR-TB. Like INH, ETH is also thought to be a pro-drug that is activated by bacterial metabolism. The activated drug then disrupts cell wall biosynthesis by inhibiting mycolic acid synthesis (Johnson *et al*., 2009).

Mutations in *ethA* and *inhA* confer resistance to ethionamide. Furthermore, co-resistance to isoniazid and ethionamide can be mediated by mutations that alter the *InhA* target or cause their over expression, or by mutations in *ndh* that increase the intracellular concentration of NADH (Vilche`ze *et al*., 2006; Pedro and Juan, 2011).

Capreomycin

Capreomycin is a macrocyclic polypeptide antibiotic isolated from *Streptomyces capreolus* (Karakousis, 2009). Capreomycin, like streptomycin and kanamycin, inhibits protein synthesis through modification of ribosomal structures at the 16S rRNA. Recent studies using site-directed mutagenesis have identified the binding site of capreomycin on 16S rRNA helix 44 (Akbergenov and Shcherbakv *et al*. 2011; Anastasia and Petros, 2012)**.** Kanamycin is aminoglycoside antibiotics. It is used as second-line drug in the treatment of MDR-TB. Its activity is at the level of protein translation (Pedro and Juan, 2011). Mutations in the gene *tlyA* have been implicated in resistance to capreomycin. This gene codes an rRNA methyltransferase specific for 2′-O-

methylation of ribose in rRNA. When mutated, it determines an absence of methylation activity. Interestingly, resistance to ribosome-targeting drugs is generally associated with the addition of methyl groups to rRNA rather than their loss (Johansen et al., 2006). However, other more recent studies have not found any mutations in *tlyA* in CAP-resistant strains (Pedro and Juan, 2011).

In *M. tuberculosis*, resistance to capreomycin and kanamycin has been associated with mutations in the *rrs* gene encoding 16S rRNA. Mutations in the gene *tlyA* encoding a 2'-O-methyltransferase of 16S rRNA and 23S rRNA have been implicated in resistance to capreomycin and viomycin and such resistance is generally associated with the addition of methyl groups to rRNA rather than their loss. However, recent studies have shown that capreomycin-resistant strains lack mutations in *tlyA* (Anastasia and Petros, 2012).

Cycloserine

D-cycloserine is an analogue of D-alanine that inhibits the synthesis of peptidoglycan by blocking the action of D-alanine: D-alanine ligase (Ddl). It also inhibits D-alanine racemase (Alr) involved in the interconversion of L-alanine and D-alanine, which then serves as a substrate for Ddl (Pedro and Juan, 2011).

Over expression of *M. tuberculosis AlrA* and Ddl on a multicopy vector results in resistance to D-cycloserine in *M. smegmatis* and *M. bovis* BCG, although whether similar mechanisms are responsible for cycloserine resistance in *M. tuberculosis* remain to be determined (Anastasia and Petros, 2012).

Para-aminosalicylic acid (PAS)

P-amino salicylic acid was one of the first antibiotics to show anti-TB activity and was used in the treatment of the disease in combination with isoniazid and streptomycin (Pedro and Juan, 2011). PAS is thought to inhibit folic acid biosynthesis and uptake of iron (Anastasia and Petros, 2012).

Its mechanism of action was never clearly elucidated. It was suggested that it may compete with para-amino benzoic acid for dihydropteroatesynthase, an enzyme needed in folate biosynthesis (Pedro and Juan, 2011). However, only slightly more than a third of the evaluated PAS-resistant strains had mutations in *thyA*, suggesting the existence of additional mechanisms of PAS resistance. *Thr202Ala* has been reported as the most common mutation associated with PAS resistance (Anastasia and Petros, 2012).

New drugs, new targets and new resistance mechanisms

Several new drugs have emerged recently as potential candidates for the treatment of TB. In most cases, their mechanism of action is distinct from that of the classical anti-TB drugs. Some of the new anti-TB drugs and its mechanism of action and resistance mechanism are discussed bellow (Anastasia and Petros, 2012).

Nitroimidazoles

Reduced oxygen tension may be an important micro environmental condition encountered by persistent bacilli within necrotic lung granulomas in the human host (Anastasia and Petros, 2012). The compounds PA-824, a nitroimidazo-oxazine, and OPC-67683, a nitroimidazo-oxazole, have shown activity against *M. tuberculosis* strains susceptible and resistant to classical anti-TB drugs (Rivers and Mancera, 2008; Pedro and Juan, 2011).

MIC values reported for PA-824 are in the range of 0.015– 0.25 mg/L for drug-susceptible strains and 0.03–0.53 mg/L for drug-resistant strains (Ginsberg *et al*., 2009) PA-824 has also shown activity against anaerobic non-replicating bacilli. PA-824 is a pro-drug that needs to be metabolized by *M. tuberculosis* in order to be activated, involving a bioreduction of the aromatic nitro group to a reactive nitro radical anion intermediate within the cell (Pedro and Juan, 2011).

The mechanism of action has been found to be inhibition of cell wall lipid and protein synthesis; although its activity against non-replicating bacteria shows that cell wall biosynthesis inhibition is probably not the only mode of action (Pedro and Juan, 2011).

It was found that resistance was mediated by the loss of a specific glucose-6-phosphate dehydrogenase or its deazaflavin cofactor F_{420}, which could provide electrons for reduction. OPC-67683, on the other hand, has shown MIC values of 0.006–0.024 mg/L with no cross-resistance with first-line drugs. The mode of action of OPC-67683 is by inhibition of the synthesis of methoxy- and keto-mycolic acids, but not alphamycolic acid (Pedro and Juan, 2011).

SQ109

SQ109 was identified by screening a large synthesized combinatorial library based on the 1, 2-ethylenediamine structure of EMB, and was found to have limited toxicity and potent activity against intracellular bacilli as well as in a murine model of chronic TB infection potential to enhance the treatment of TB during the first 2 months of intensive therapy and also to treat MDR-TB (Lalloo and Ambaram 2010; Anastasia and Petros, 2012). It has shown good activity, with MIC values ranging from 0.16 to 0.63 mg/L, and intermediate cytotoxicity assessed in cell viability assays (Pedro and Juan, 2011).

The mode of action of SQ109 is not well known, but it is believed that it affects mycobacterial cell wall synthesis in a different manner to that exerted by ethambutol. It has been found that in strains resistant to isoniazid, ethambutol and SQ109, there is up-regulation of *ahpC*, suggesting a possible role in the development of resistance to this drug (Jia *et al*., 2005).

NAS-21 and NAS-91

The anti-malarial agents NAS-21 and NAS-91 have recently been shown to have potent antimycobacterial activity, inhibiting mycolic acid biosynthesis and profoundly altering the production of oleic acids (Gratraud *et al*., 2008). In studies with *M. bovis* BCG, it has been suggested that the main target could be the FAS-II dehydratase coded by *Rv0636*. It has been shown that strains resistant to these compounds over express Rv0636 gene analogues (Pedro and Juan, 2011).

Benzothiazinones

The 1, 3-benzothiazin-4-ones (BTZs) represent a new class of drugs, which have activity against *M. tuberculosis in vitro, ex vivo*, and in murine TB models (Makarov and Manina, 2009). BTZs are activated in *M. tuberculosis* by reduction of an essential nitro group to a nitroso derivative, which then specifically reacts with a cysteine residue in the active site of the enzyme decaprenylphosphoryl-B-D-ribose 2'-epimerase (DprE1) (Trefzer and Rengifo, 2010). Inhibition of this enzymatic activity abolishes the formation of decaprenylphosphoryl arabinose, a key precursor that is required for the synthesis of the cell-wall arabinans, thus causing bacterial lysis and death (Makarov and Manina, 2009). Recently, a novel resistance mechanism to BTZ was described in *M. smegmatis* involving the over expression of the nitroreductase *NfnB*, which leads to the inactivation of the drug by reduction of a critical nitro-group to an amino-group. However, *M. tuberculosis* seems to lack nitroreductases able to inactivate BTZs (Anastasia and Petros, 2012).

Conclusion

Even though mutations in several genes are evidently related to drug resistance in *M. tuberculosis*, there are numerous resistant strains that do not present these classic mutations. Some characteristics of TB treatment such as defaulting from treatment are well known predictors of multidrug resistance, other aspects of treatment such as the drugs used and the length of treatment need to be studied as they may contribute to improve control program. Novel anti-TB drugs, which are safe, able to shorten the course of treatment, effective against drug-resistant strains and latent TB infection, are urgently needed, especially in the era of MDR- and XDR-TB. Regarding the dynamics of TB transmission, and also in view of the need to develop new anti-TB drugs, it is extremely important to further our knowledge on the molecular basis of drug resistance and all its complexity. To meet the challenge of MDR-TB and XDR-TB worldwide, huge monetary resource instillation and extensive human resource development are required. In summary, even if there has been growing concern among clinicians, epidemiologists and public health workers worldwide, further researches must be conducted in the areas of molecular biology. Application of basic knowledge in the field of epidemiology can help understand the mechanisms of MDR-*Mycobacterium TB.*

Conflict of interest statement

Authors declare that they have no conflict of interest.

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