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Review Article

Multidrug Resistance in Tuberculosis: An Overview

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Abstract

Mycobacterium tuberculosis is an extraordinarily successful human pathogen, infecting one-third of the world's population and causing nearly two million deaths each year. In this article, current trends in worldwide tuberculosis (TB) resistance are discussed along with pathogenesis of drug resistance, emergence of resistance, mechanism of resistance development, prevention of drug resistance. The global TB emergency has been further exacerbated by multi drug-resistant (MDR) TB and and extensively drug-resistant (XDR) TB strains that are resistant to our best antibiotics and very difficult to treat.

1. INTRODUCTION

Mycobacterium tuberculosis is infecting one third of the global population. Currently, tuberculosis management and control is potentially devastating threat worldwide due to emergence of drug resistant strains. The modern, standard short-course therapy for TB recommended by the World Health Organization is based on a four-drug regimen that relies on direct observation of patient compliance to ensure effective treatment. The rapid spread of drug resistance especially multi-drug resistant tuberculosis (MDR-TB) and currently extensively drug resistant tuberculosis (XDR-TB), both in new and previously treated cases, adds urgency to the need for decisive action for control measures . Resistant to at least two major anti tuberculosis drugs; Isoniazid and Rifampicin with or without resistance to other anti-TB drugs has been termed MDR-TB. MDR-TB is more difficult to treat than drug- susceptible TB, requiring the use of less effective second line anti tubercular drugs (SLDs) which are often associated with major side effects.

The present review gives a concise summary of the mechanism of action and molecular basis of resistance. In addition, the how to prevent the drug resistant was emphasized.

Tuberculosis, MTB, or TB (short for *tubercle bacillus*), in the past also called phthisis, phthisis pulmonalis, or consumption, is a common, and in many cases fatal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*[1] Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit respiratory fluids through the air[2]. Most infections do not have symptoms, known as latent tuberculosis. About one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected.

The classic symptoms of active TB infection are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss (the latter giving rise to the formerly common term *consumption*). Infection of other organs causes a wide range of symptoms. Diagnosis of active TB relies on radiology (commonly chest X-rays), as well as microscopic examination and microbiological culture of body fluids. Diagnosis of latent TB relies on the tuberculin skin test (TST) and/or blood tests. Treatment is difficult and requires administration of multiple antibiotics over a long period of time. Social contacts are also screened and treated if necessary. Antibiotic resistance is a growing problem in multiple drug-resistant tuberculosis(MDR-TB) infections. Prevention relies on screening programs and vaccination with the bacillus Calmette–Guérin vaccine.

One third of the world's population is thought to have been infected with *M. tuberculosis*[3] with new infections occurring in about 1% of the population each year[4]. In 2007, there were an estimated 13.7 million chronic active cases globally [5]. While in 2010, there were an estimated 8.8 million new cases and 1.5 million associated deaths, mostly occurring in developing countries[6]. The absolute number of tuberculosis cases has been decreasing since 2006, and new cases have decreased since 2002. The rates of tuberculosis in different areas varies across the globe; about 80% of the population in many Asian and African countries tests positive in tuberculin tests, while only 5–10% of the United States population tests positive . More people in the developing world contract tuberculosis because of a poor immune system, largely due to high rates of HIV infection and the corresponding development of AIDS [7].

2. SIGNS AND SYMPTOMS

The main symptoms of variants and stages of tuberculosis are given[8] with many symptoms overlapping with other variants, while others are more (but not entirely) specific for certain variants. Multiple variants may be present simultaneously.

Tuberculosis may infect any part of the body, but most commonly occurs in the lungs (known as pulmonary tuberculosis) [9]. Extra-pulmonary TB occurs when tuberculosis develops outside of the lungs, although extra-pulmonary TB may coexist with pulmonary TB as well.

General signs and symptoms include fever, chills, night sweats, loss of appetite, weight loss, and fatigue. Significant finger clubbing may also occur.

3. PREVENTION OF TB IN CHILDREN

3.1 The Three I's

It is more cost effective to prevent disease than it is to treat it. The most effective way to prevent TB is to stop the disease from spreading. This is achieved by what is commonly referred to as the

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Three I's — intensified case finding, isoniazid preventive therapy, and infection control.

Intensified case finding
Finding and treating adults with TB is not enough to help children. When an adult is diagnosed with TB, all close contacts and family members — including children — should be screened and, if symptomatic, provided appropriate diagnosis and treatment. Additionally, children at high risk of TB, including those living with HIV, should be routinely screened. A large proportion of childhood TB cases could be prevented by treating infected children discovered during case finding.

Isoniazid preventive therapy (IPT)
All asymptomatic children exposed to an adult with TB should be provided IPT, which prevents infection from developing into active disease. IPT is especially important for children diagnosed with HIV.

Infection control
It is likely that TB is spread within many healthcare facilities in high-burden areas. Therefore, it is extremely important that health facilities, homes, schools, and other community settings need to be made safe from TB. Simple measures such as separating patients who are coughing, providing masks, and opening windows and doors to establish natural ventilation can prevent the spread of disease.
These methods are very effective at reducing childhood TB and are endorsed by the WHO. A recent study from Zambia and South Africa found children living in communities that engaged in intensified case finding were 50 percent less likely to become infected with TB. Unfortunately, many countries with constrained resources don't follow these methods. Increased resources, training, and health care workers are needed to make TB prevention a reality. Donor governments should continue to invest in TB treatment and prevention programs through bilateral TB programs; the Global Fund to Fight AIDS, Tuberculosis and Malaria; and UNITAID. High-burden countries must also increase national TB budgets and consider alternate funding revenues to fund TB services, such as a national tobacco tax or other taxes.

The fourth I: Integration
Integration of TB services — often referred to as the *'fourth I'* — is central to tackling HIV and improving maternal and child health. HIV weakens the immune system, making a person vulnerable to TB. Nearly half of new childhood TB cases occur in children with HIV; TB remains the third leading killer of children with AIDS[10]. Historically, TB prevention, treatment, and diagnosis have not been included with other child health services.
To ensure more children receive TB services, the following must be implemented:
Health care workers must be trained and supported to address childhood TB and TB services must be incorporated into the Integrated Management of Childhood Illnesses (IMCI), a broad child health strategy that includes multiple interventions at health facilities and in communities. TB services should be incorporated with maternal health care and the prevention of mother-to-child transmission of HIV (PMTCT). The WHO and the President's Emergency Plan for AIDS Relief (PEPFAR) recommend screening all pregnant women with HIV for TB, as pregnant women with TB are 2.5 times more likely to pass on HIV to their unborn child [11,12,13]. Because children with HIV are at high risk of developing TB — up to 20 times more likely than children with healthy immune systems[14]— it is imperative that all children with HIV are screened for TB at every health care visit, and that all children with TB are screened for HIV.
Once diagnosed, children living with HIV should be placed on ART immediately. Early initiation of ART is the single most important intervention for reducing overall mortality and the risk of TB among HIV-infected infants, reducing the chances of getting TB by 70 percent.

4. WHAT DO YOU MEAN BY 'RESISTANT'?

The term 'drug resistance' is ambiguously defined in many situations. What is drug resistance, especially in the context of M. tuberculosis? The WHO defines drug resistance as ''the ability of certain microorganisms to withstand attack by antimicrobials.'' In the context of *M. tuberculosis*, this is defined as the ability of >1% proportion of a bacilli to grow in the presence of critical concentration of drug. The critical concentrations themselves are defined as the concentration of antibiotic that inhibit growth in 95% of wild type strains that have hitherto not been exposed to drug. Thus, these are essentially epidermiologic cut-off values[15]. Antibiotics have a long history, beginning in the 1930s and earlier, during which several distinct drug classes were discovered and numerous improved analogs were made available[16]. Because of these efforts, today's antibiotics satisfactorily address most clinical situations; the escalating multidrug resistance problem is a major exception. Therefore, resistance remains as a primary driver for antibacterial R&D. Indeed, there is little economic and medical justification for the development of new antibiotics that do not solve relevant resistance problems. Without resistance the future of antibacterial R & D would be limited[17].

4.1 Molecular mechanisms of drug resistance

In order to control the drug resistance epidemic it is necessary to gain insight into how M. tuberculosis develops drug resistance. This knowledge will help us to understand how to prevent the occurrence of drug resistance as well as identifying genes associated with drug resistance of new drugs. 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. 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 INH and 3.1 × 10–8 for RIF. Because the chromosomal loci responsible for resistance to various drugs are not linked, the risk of a double spontaneous mutation is extremely low: 9 × 10–14 for both INH and Rif[18]. MDR-TB defined as resistance to at least INH and RIF will thus occur mainly in circumstances where sequential drug resistance follows sustained treatment failure. Treatment can be divided into first line and second line drugs according to the WHO TB treatment regimen and the mechanisms of these will be discussed separately.

4.2 Pathogenesis of drug resistance

In every 106 to 108 replications, wild strains of *MTB* undergo spontaneous mutations that confer resistance to a single drug; the average number of such spontaneous mutations to anti-TB drugs is shown. (Table 1) [19,20] When treated with a single drug, the population of TB bacilli initially shrinks due to the killing of susceptible organisms in the population, often rendering a person smear-negative (as a result of fewer organisms being present). However, the organisms that survive the initial phase are the drug resistant mutants, and the proliferation of these mutants eventually causes the entire population of bacilli to be replaced by drug-resistant forms that continue to proliferate until they are numerous enough to cause recurrence of symptoms, and smear positivity; this is termed "the fall and rise phenomenon"[21]. If treated with a single drug, and the bacillary load of the organisms exceeds 106, then emergence of strains that are resistant to that drug is almost certain. If the bacillary load exceeds 108 then resistance is likely to develop if only two drugs are used. Bacillary loads exceed 106 with tuberculous infiltrates alone (when sputum direct smears are negative although cultures are positive), and exceed 108 when cavities are present in patients with TB, at which time sputum direct smears are usually positive [22,23]

Table 1: Drug versus average muatation rate

Drug	Average mutation rate
Isoniazid	2.56×10^{-8}
Rifampicin	2.25×10^{-10}
Ethambutol	1×10^{-7}
Streptomycin	2.95×10^{-5}
Pyrazinamide	1×10^{-6}

One of the aims of modern anti-TB therapy is to prevent drug resistant mutants from proliferating. This is best accomplished by including at least three likely effective anti-tuberculous agents in the initial treatment regimen, as this will reduce the probability of emergence of drug resistance to 10-18 or lower. During the initial phase of treatment the few mutants with spontaneous resistance to one drug will be killed more slowly than the "wild type" bacilli that

are susceptible to all drugs. Hence during the first months of therapy these more resistant bacilli will survive longer. If therapy is interrupted early, through default, then these drug-resistant mutants will proliferate, increasing the proportion of drugresistant forms, until this proportion becomes clinically significant. Low drug levels, either from malabsorption (as occurs in HIV-infected patients) or inadequate dosages of medications, will have the same effect.

5. FIRST LINE DRUGS USED IN TB TREATMENT

Any drug used in the anti-TB regiment is supposed to have an effective sterilizing activity that is capable of shortening the duration of treatment. Currently, a four-drug regiment is used consisting of INH, RIF, pyrazinamide (PZA) and ethambutol (EMB). Resistance to first line anti-TB drugs has been linked to mutations in at least 10 genes; katG, inhA, ahpC, kasA and ndh for INH resistance; rpoB for RIF resistance, embB for EMB resistance, pncA for PZA resistance and rpsL and rrs for STR resistance. Isoniazid KatG. INH or isonicotinic acid hydrazide, was synthesized in the early 1900s but its anti-TB action was first detected in 1951[24]. INH enters the cell as a prodrug that is activated by a catalase peroxidase encoded by katG. The peroxidase activity of the enzyme is necessary to activate INH to a toxic substance in the bacterial cell[25]. This toxic substance subsequently affects intracellular targets such as mycolic acid biosynthesis which are an important component of the cell wall. A lack of mycolic acid synthesis eventually results in loss of cellular integrity and the bacteria die. Middlebrook et al. initially demonstrated that a loss of catalase activity can result in INH resistance[26]. Subsequently genetic studies demonstrated that transformation of INH-resistant Mycobacterium smegmatis and M. tuberculosis strains with a functional katG gene restored INH susceptibility and that katG deletions give rise to INH resistance. However, mutations in this gene are more frequent than deletions in clinical isolates and these can lower the activity of the enzyme. Most mutations are found between codons 138 and 328 with the most commonly observed gene alteration being at codon 315 of the katG gene[27]. The Ser315Thr substitution is estimated to occur in 30–60% of INH resistant isolates.

The katG 463 (CGG-CTG) (Arg-Leu) amino acid substitution is the most common polymorphism found in the katG gene and is not associated with INH resistance.ahpC. It has been observed that a loss of katG activity due to the S315T amino acid substitution is often accompanied by an increase in expression of an alkyl hydroperoxide reductase (ahpC) protein that is capable of detoxifying damaging organic peroxides. Five different nucleotide alterations have been identified in the promoter region of the ahpC gene, which lead to over expression of ahpC and INH resistance[28]. AhpC overexpression exerts a detoxifying effect on organic peroxides within the cell and protects the bacteria against oxidative damage but does not provide protection against INH. KatG expression can also be up regulated under conditions of oxidative stress. The correlation between polymorphic sites in the ahpC regulatory region with INH resistance in M. tuberculosis requires further examination.

5.1 inhA.

One of the targets for activated INH is the protein encoded by the inhA locus. InhA is an enoyl–acyl carrier protein (ACP) reductase which is proposed to be the primary target for resistance to INH and ethionamide (ETH)[29]. ETH, a second line drug, is a structural analog of INH that is also thought to inhibit mycolic acid biosynthesis and several studies have suggested that low-level INH resistance is correlated with resistance to ETH. Activated INH binds to the InhA-NADH complex to form a ternary complex that results in inhibition of mycolic acid biosynthesis. Six point mutations associated with INH resistance within the structural inhA gene have been identified (Ile16Thr, Ile21Thr, Ile21Val, Ile47Thr, Val78Ala and Ile95Pro)[30]. A Ser94Ala substitution results in a decreased binding affinity of inhAfor NADH, resulting in mycolic acid synthesis inhibition. Although these mutations in the structural InhA gene are associated with INH resistance, it is not frequently reported in clinical isolates.

InhA promoter mutations are more frequently seen and are present at positions -24(G-
T), -16(A-G), or -8(T-G/A) and -15(C-T). These promoter mutations result in over expression of inhA leading to low level INH resistance. To date approximately 70–80% of INH resistance in clinical isolates of M. tuberculosiscan be attributed to mutations in the katG and inhA genes.

5.2 kasA.

There seems to be considerable dispute within the literature as to the role of kasA as a possible target for INH resistance[31]. This gene encodes a β-ketoacyl-ACP synthase involved in the synthesis of mycolic acids. Mutations have been described in this gene that confer low levels of INH resistance. Genotypic analysis of the kasA gene reveals 4 different amino acid
substitutions involving codon 66 (GAT-AAT), codon 269 (GGT-AGT), codon 312 (GGC-AGC) and codon 413 (TTC-TTA). However, similar mutations were also found in INH susceptible isolates[32]. Nevertheless, the possibility of kasA constituting an additional resistance mechanism should not be completely excluded.ndh. In 1998 another mechanism for INH resistance was described by Miesel et al.[33]. The ndh gene encodes NADH dehydrogenase that is bound to the active site of inhA to form the ternary complex with activated INH. Structural studies have shown that a reactive form of INH attacks the NAD(H) co-factor and generates a covalent INH-NAD adduct. Mutations in the ndh gene, encoding NADH dehydrogenase, cause defects in the enzymatic activity. Thus, defects in the oxidation of NADH to NAD result in NADH accumulation and NAD depletion. These high levels of NADH can then inhibit the binding of the INH-NAD adduct to the active site of the InhA enzyme. Prominent point mutations in the ndh gene at codons 110 and 268 (T110A and R268H) were detected in 9.5% of INH resistant samples. These similar mutations were not detected in the INH susceptible group [34].

5.3 Rifampicin

RIF was fist introduced in 1972 as an anti-TB drug and has excellent sterilizing activity [35]. The action of RIF in combination with PZA has allowed a shortening of routine TB treatment from 1 year to 6 months. RIF in combination with INH forms the backbone of short-course chemotherapy. It is interesting to note that mono resistance to INH is common but mono resistance to RIF is quite rare. It has thus been proposed that resistance to RIF can be used as a surrogate marker for MDR-TB as nearly 90% of RIF resistant strains are also INH resistant [36]. RIF interferes with transcription by the DNA-dependent RNA polymerase. RNA polymerase is composed of four different subunits (α, β, β' and σ) encoded by rpoA, rpoB, rpoC and rpoD genes respectively. RIF binds to the β-subunit hindering transcription and thereby killing the organism. Extensive studies on the rpoB gene in RIF resistant isolates of M. tuberculosis identified a variety of mutations and short deletions in the gene. A total of 69 single nucleotide changes; 3 insertions, 16 deletion and 38 multiple nucleotide changes have been reported[37]. More than 95% of all missense mutations are located in a 51bp core region (Rifampicin resistance determining region) of the rpoB gene between codons 507–533 with the most common changes in codons Ser531Leu, His526Tyr and Asp516Val. These changes occur in more than 70% of RIF resistant isolates. Furthermore, the minimal inhibitory concentration (MIC) showed that high level of RIF resistance is associated with mutations in codon 526 and 531, whereas alterations in codon 511,516, 518 and 522 result in low level RIF resistance.

5.4 Pyrazinamide

PZA, a nicotinamide analog, was first discovered to have anti-TB activity in 1952. PZA targets an enzyme involved in fatty-acid synthesis and is responsible for killing persistent tubercle bacilli in the initial intensive phase of chemotherapy. However, during the first two days of treatment, PZA has no bactericidal activity against rapidly growing bacilli[38]. PZA on the other hand has effective sterilizing activity and shortens the chemotherapeutic regiment from 12 to 6 months. PZA is a prodrug which is converted to its active form, pyrazinoic acid (POA) by the pyrazinamidase (PZase) encoded by pncA. The activity of PZA is highly specific for M. tuberculosis, as it has no effect on other mycobacteria. Mycobacterium bovis is naturally resistant to PZA due to a unique C-G point mutation in codon 169 of the pncA gene. PZA is only active against M. tuberculosis at acidic pH where POA accumulates in the cytoplasm due to an ineffective efflux pump. Accumulation of POA results in the lowering of intracellular pH to a level that inactivates a vital fatty acid synthase[39]. Cloning and

characterization of the M. tuberculosis pncA gene by Scorpio et al.[40] showed that pncA mutations conferred PZA resistance. Various pncA mutations have been identified in more than 70% of PZA resistant clinical isolates scattered throughout the pncA gene but thus far no mutational hot spot has been identified. In a study from Peru it was found that 59% of MDR patients also had M. tuberculosis resistant to PZA. PZA susceptibility testing is not done routinely in many countries due to technical difficulties. Thus the extent of PZA resistance globally is largely unknown. A study done by Louw et al.[41] showed that PZA resistance is common amongst drug-resistant clinical M. tuberculosis isolates from South Africa. PZA resistance was shown to be strongly associated with MDR-TB and therefore it was concluded that PZA should not be relied upon in managing patients with MDR-TB in this setting. PZA resistant isolates had diverse nucleotide changes scattered throughout the pncA gene. Mutations in the pncA gene correlate well with phenotypic resistance to PZA. However, PZA resistant isolates without pncA mutations were also observed suggesting that another mechanism may be involved in conferring PZA resistance in these isolates. In addition, not all mutations (e.g. Thr114Met) were associated with PZA resistance. In summary, the complexity of PZA resistance makes the development of molecular methods for rapid diagnosis difficult.

5.5 Ethambutol

EMB, a first line drug, is used in combination with other drugs and is specific to the mycobacteria. EMB inhibits an arabinosyl transferase (embB) involved in cell wall biosynthesis [42]. Telenti et al. identified 3 genes, designated embCAB, that encode homologous arabinosyl transferase enzymes involved in EMB resistance. Various studies have identified five mutations in codon 306 [(ATG-GTG), (ATG-CTG), (ATG-ATA), (ATG-ATC) and (ATG-ATT)] which result in three different amino acid substitutions (Val, Leu and Ile) in EMB-resistant isolates . These five mutations are associated with 70–90% of all EMB resistant isolates. Missense mutations were identified in three additional codons: Phe285leu, Phe330Val and Thr630Ile in EMB resistant isolates. MIC's were generally higher for strains with Met306Leu, Met306Val, Phe330Val and Thr630Ile substitutions than those organisms with Met306Ile substitutions. Mutations outside of codon 306 are present but quite rare. In a study recently done by Johnson et al[43] it was shown that genotypic analysis identified mutations at codon 306 of the embB gene rendering resistance to EMB. However, routine phenotypic analysis failed to identify EMB resistance in 91.4% of resistant isolates in this setting and confirm the difficulty of EMB phenotypic testing. The inability to accurately detect true EMB resistance by the culture based method have a negative impact on the TB control program. Molecular-based methods offers a rapid diagnosis of EMB resistance and could thereby benefit the management of TB patients within days. However a number of EMB phenotypic resistant isolates (about 30%) still lack an identified mutation in embB. There is therefore a need to fully understand the mechanism of EMB resistance in clinical isolates.

5.6 Streptomycin

STR, an aminocyclitol glycoside, is an alternative first line anti-TB drug recommended by the WHO. STR is therefore used in the retreatment of TB cases together with the four drug regimen that includes INH, RIF, PZA and EMB[44]. The effect of STR has been demonstrated to take place at the ribosomal level. STR interacts with the 16S rRNA and S12 ribosomal protein (rrs and rpsL), inducing ribosomal changes, which cause misreading of the mRNA and inhibition of protein synthesis. Although STR is a recommended anti-TB drug, is it less effective against M. tuberculosis than INH and RIF. Point mutations in STR resistant isolates have been reported in rrs and rpsL genes in 65–67% of STR resistant isolates. In the rrs gene a C-T transition at positions 491, 512 and 516, and a A-C/T transversion at position 513 were observed in the highly conserved 530 loop. The 530 loop region is part of the aminoacyl–tRNA binding site and is involved in the decoding process. The C-T transition at codon 491 is not responsible for resistance to STR as it occurs in both STR resistant and susceptible isolates but is strongly associated with the global spread of M. tuberculosis with a Western Cape F11 genotype[45]. Other mutations in the 915 loop [903 (C-A/G) and 904 (A-G)] have also been reported to have an association with STR resistance.

6. SECOND LINE DRUGS USED IN TB TREATMENT

According to the WHO the following drugs can be classified as second line drugs: aminoglycosides (kanamycin and amikacin) polypeptides (capreomycin, viomycin and enviomycin), fluoroquinolones (ofloxacin, ciprofloxacin, and gatifloxacin), D-cycloserine and thionamides (ethionamide and prothionamide)[46]. Unfortunately, second-line drugs are inherently more toxic and less effective than first-line drugs. Second line drugs are mostly used in the treatment of MDR-TB and as a result prolong the total treatment time from 6 to 9 months [47]. The current understanding of molecular mechanisms associated with resistance to second line drugs are summarized in Table 3. The phenotypic methods to detect resistance to second line drugs are less well established and the molecular mechanisms of resistance are also less defined.

6.1 Fluoroquinolones

Ciproflaxin (CIP) and ofloxacin (OFL) are the two fluoroquinolones (FQs) used as second-line drugs in MDR-TB treatment. The quinolones target and inactivate DNA gyrase, a type II DNA topoisomerase[48]. DNA gyrase is encoded by gyrA and gyrB and introduces negative supercoils in closed circular DNA molecules. The quinolone resistance-determining region (QRDR) is a conserved region in the gyrA (320bp) and gyrB (375bp) genes[49] which is the point of interaction of FQ and gyrase. Missense mutations in codon 90, 91, and 94 of gyrA are associated with resistance to FQs. A 16-fold increase in resistance was observed for isolates with a Ala90Val substitution, a 30-fold increase for Asp94Asn or His94Tyr and a 60-fold increase for Asp94Gly. A polymorphism at gyrA codon 95 is not associated with FQ resistance, and is used, with the katG463 polymorphism, to classify M. tuberculosis into 3 phylogenetic groups.

6.2 Aminoglycosides

Kanamycin (KAN) and Amikacin (AMI) are aminoglycosides which inhibit protein synthesis and thus cannot be used against dormant M. tuberculosis. Aminoglycosides bind to bacterial ribosomes and disturb the elongation of the peptide chain in the bacteria. Mutations in the rrs gene encoding for 16s rRNA are associated with resistance to KAN and AMI. Nucleotide changes at positions 1400, 1401 and 1483 of the rrs gene have been found to be specifically associated with KAN resistance[50]. An A→G change at codon 1400 in the rrs gene showed resistance to KAN of MICs more that 200 µg/ml.

6.3 Ethionamide

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 that is activated by bacterial metabolism. The activated drug then disrupts cell wall biosynthesis by inhibiting mycolic acid synthesis. Mutations in the promoter of the inhA gene are associated with resistance to INH and ETH [51].

6.4 D-Cycloserine

D-cycloserine (DCS) is a cyclic analog of D-alanine which is one of the central molecules of the cross linking step of peptidoglycan assembly. DCS inhibits cell wall synthesis by competing with D-Alanine for the enzymes D-alanyl-D-alanine synthetase (Ddl) and D-alanine racemase (Alr) and also inhibiting the synthesis of these proteins. Over expression of alr cause DCS resistance. A G→T transversion in the alr promoter may lead to the overexpression of alr [52].

6.5 Peptides

Viomycin (VIO) and capreomycin (CAP) are basic peptide antibiotics that inhibit prokaryotic protein synthesis and are used as second-line anti-TB drugs. Earlier studies have shown that resistance to VIO in M. smegmatis is caused by alterations in the 30S or 50S ribosomal subunits. Mutations in the rrs gene that encodes the 16S rRNA is associated with resistance to VIO and CAP, specifically a G→A or G→T nucleotide change at codon 1473[53].

7. MOLECULAR METHODS TO PREDICT DRUG RESISTANCE

1) Sequencing: PCR amplification followed by DNA sequencing is the most widely used
technique to identify mutations associated with drug resistance in TB [54]. This technique is costly and require expertise, which make it unpractical for use in routine laboratories.

2) Probe-based hybridization methods: In these assays, amplified PCR products of genes known to confer drug resistance are hybridized to an allele-specific labeled probe that is complementary to the wild type or mutant sequence of the gene. This can then be visualized by autoradiography.

3) PCR restriction fragment length polymorphism (PCR-RFLP): Mutations associated with resistance can be identified by digestion of amplified PCR products with a restriction enzyme that cuts at the specific polymorphic DNA sequence followed by gel electrophoresis.

4) Single stranded conformation polymorphism analysis (SSCP): SSCP is a gel based method that can detect short stretches of DNA approximately 175–250bp in size. Small changes in a nucleotide sequence result in differences in secondary structures as well as measurable DNA mobility shifts that are detected on a non-denaturing polyacrylamide gel. To date various studies have applied PCR-SSCP to identify mutational changes associated with drug resistance in M. tuberculosis for frontline drugs like, RIF and INH [55].

5) Heteroduplex analysis (HA): HA depends on the conformation of duplex DNA when analysed in native gels. Heteroduplexes are formed when PCR amplification products from known wild type and unknown mutant sequences are heated and re-annealed. The DNA strand will form a mismatched heteroduplex if there is a sequence difference between the strands of the wild type and tested DNA. Recently, temperature mediated HA has been applied to the detection of mutations associated with mutations in rpoB, katG, rpsL, embB and pncA genes [56].

6) Amplification refractory mutation system (ARMS)-PCR : ARMS also known as allelic specific PCR (ASPCR) or PCR amplification of specific alleles (PASA) is a well established technique used for the detection of any point mutation or small deletions [57].

7) Molecular beacons : Molecular beacons are single-stranded oligonucleotide hybridization probes which can be used as amplicon detector probes in diagnostic assays. Molecular beacons are very specific and can discriminate between single nucleotide substitutions. Thus they are ideally suited for genotyping and have been used in the detection of drug resistance in M. tuberculosis [58].

8. HOW TO PREVENT DRUG RESISTANCE?

The broad objectives of anti-TB treatment are:

(1) Rapid reduction in bacillary load to reduce morbidity and mortality, and stop transmission.
(2) Prevent the emergence of drug resistant mutant strains, and
(3) Prevent relapse of disease.

To achieve objective 1potent bactericidal drugs such as isoniazid, especially in the first week and rifampicin are the most useful.

To achieve the second objective, multiple drugs with proven (by DST) or likely (never previously used) efficacy are used to prevent the selection of drug-resistant mutants as explained earlier.

To achieve the third objective, treatment is prescribed for a sufficiently long duration, with monitoring of adherence to treatment, to eliminate residual surviving organisms that are responsible for disease relapse. The length of treatment with rifampicin plays an important role in achieving this third objective. Recommendations for the dosages, duration, and Combinations of drugs for treatment of drug- suscetible TB are based on sound evidence based principles derived from multiple randomized trials. Adherence to authoritative guidelines for treatment and ensuring that all doses are taken correctly is unarguably the most effective means of preventing drug resistance.

9. CONCLUSION

Pathogenic organisms, such as M. tuberculosis, that significantly contribute to worldwide human infectious disease are also the most common antibiotic-resistant bacteria. Our arsenal of antimicrobials is currently under attack by micro organisms themselves as clinically significant, antibiotic-resistant bacteria evolve at alarming rates. The fight against antibiotic resistance is formidable, but must be endeavoured in the face of treatment failures, prolonged illnesses, increased deaths, and escalated risks of infections. With increase in worldwide cases of MDR- and XDR-TB occurring on a yearly basis, the grim progression from antibiotic effectiveness to antibiotic resistance drives this global crisis.

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REFERENCES

1. Kumar V, Abbas AK, Fausto N, Mitchell RN (2007).*Robbins Basic Pathology* (8th ed.). Saunders Elsevier. ISBN 978-1-4160-2973-1, 516-522.
2. Konstantinos A (2010). "Testing for tuberculosis". *Australian Prescriber* **33** (1): 12–18.
3. "Tuberculosis Fact sheet N°104". World Health Organization. November 2010. Retrieved 26 July 2011.
4. "Tuberculosis". *World Health Organization*. 2002.
5. World Health Organization (2009). "Epidemiology". *Global tuberculosis control: epidemiology, strategy, financing*. ISBN 978-92-4-156380-2 , 6-33.
6. World Health Organization (2011). "The sixteenth global report on tuberculosis".
7. Lawn, SD; Zumla, AI (2 July 2011). "Tuberculosis". *Lancet* 378 (9785): doi:10.1016/S0140-6736(10)62173-3.PMID 21420161, 57-72.
8. Schiffman G (15 January 2009). "Tuberculosis Symptoms". eMedicineHealth.
9. Dolin, [edited by] Gerald L. Mandell, John E. Bennett, Raphael (2010).*Mandell, Douglas, and Bennett's principles and practice of infectious diseases* (7th ed.). Philadelphia, PA: Churchill Livingstone/Elsevier. pp. Chapter 250. ISBN 978-0-443-06839-3.
10. UNAIDS. (2007). Report on the global AIDS epidemic. Geneva: Joint United Nations Program on HIV/AIDS.
11. World Health Organization. (2011). Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. Geneva: World Health Organization.
12. The President's Emergency Plan for AIDS Relief. (2011). PEPFAR Guidance on Integrating Prevention of Mother to Child Transmission of HIV, Maternal, Neonatal, and Child Health and Pediatric HIV Services. Washington, DC: Office of the US Global AIDS Coordinator.
13. Gupta, A. et al. (2011). Maternal tuberculosis: a risk factor for mother-to-child transmission of human immunodeficiency virus. Journal of Infectious Disease 203(3): 358-363
14. Hesseling, A.C. et al. (2009). High incidence of tuberculosis among HIV-infected infants: evidence from a South h African population-based study high lights the need for improved tuberculosis control strategies. Clinical Infectious Disease 48(1): 108-114.
15. Clinical and Laboratory Standards Institute: Susceptibility Testing Of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard. Wayne, PA: Clinical and Laboratory Standards Institute; 2003.
16. Bush K: The coming of age of antibiotics: discovery and therapeutic value. Ann N Y Acad Sci, 1213,1-4, 2010.
17. Hughes JM: Preserving the lifesaving power of antimicrobial agents. JAMA 2011 doi: 10.1001/jama.2011.1279.
18. Dooley, S.W. and Simone, P.M. (1994). The extent and management of drug-resistant tuberculosis: the American experience. Clinical tuberculosis. London: Chapman & Hall 171–189.
19. David HL. Probability distribution of drug-resistant mutants in unselected populations of Mycobacterium tuberculosis. Appl Microbiol, 20(5), 1970, 810–814.

20. Antitubercular drug resistance in the world Geneva Switzerland: World Health Organisation. 1997 WHO / IUATLD Global project on Anti tuberculosis drug resistance surveillance 1994-1997 WHO-TB-97.229
21. Espinal M. What is the “fall and rise” phenomenon and the “sequential regimen” mechanism? In: Frieden T, editor. Toman’s tuberculosis: case detection, treatment, and monitoring – questions and answers. 2nd ed.Geneva, Switzerland: World Health Organization, 200–202, 2004.
22. Canetti G. The tubercle bacillus in pulmonary lesion of man; histobacteriology and its bearing on the therapy of pulmonary tuberculosis. New York: Springer; 1955.
23. Crofton J, Mitchison DA. Streptomycin resistance in pulmonary tuberculosis. Br Med J, 2(4588), 1948, 1009–1015.
24. Heym, B., Saint-Joanis, B., and Cole, S.T. (1999). The molecular basis of isoniazid resistance in Mycobacterium tuberculosis. Tuber. Lung Dis. 79, 267–271.
25. Zhang, Y., Heym, B., Allen, B., Young, D., and Cole, S. (1992). The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature 358, 591–593.
26. Middlebrook, G. (1954). Isoniazid-resistance and catalase activity of tubercle bacilli. Am. Rev. Tuberc. 69, 471–472.
27. Slayden, R.A. and Barry, C.E., III (2000). The genetics and biochemistry of isoniazid resistance in Mycobacterium tuberculosis. Microbes. Infect. 2, 659–669.
28. Ramaswamy, S. and Musser, J.M. (1998). Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update. Tuber. Lung Dis. 79, 3–29.
29. Banerjee, A., Dubnau, E., Quemard, A., Balasubramanian, V., Um, K.S., Wilson, T., Collins, D., de Lisle, G., and Jacobs, W.R., Jr. (1994). inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science 263, 227–230.
30. Basso, L.A. and Blanchard, J.S. (1998). Resistance to antitubercular drugs. Adv. Exp. Med. Biol. 456, 115–144.
31. Sherman, D.R., Mdluli, K., Hickey, M.J., Arain, T.M., Morris, S.L., Barry, C.E., III, and Stover, C.K. (1996) Compensatory ahpC gene expression in isoniazid- resistant Mycobacterium tuberculosis. Science 272, 1641–1643.
32. Lee, A.S., Lim, I.H., Tang, L.L., Telenti, A., and Wong, S.Y. (1999). Contribution of kasA analysis to detection of isoniazid-resistant Mycobacterium tuberculosis in Singapore. Antimicrob. Agents Chemother. 43, 2087–2089.
33. Miesel, L., Weisbrod, T.R., Marcinkeviciene, J.A., Bittman, R., and Jacobs, W.R., Jr. (1998). NADH dehydrogenase defects confer isoniazid resistance and conditional lethality in Mycobacterium smegmatis. J. Bacteriol. 180, 2459–2467.
34. Lee, A.S., Teo, A.S., and Wong, S.Y. (2001). Novel mutations in ndh in isoniazid-resistant Mycobacterium tuberculosis isolates. Antimicrob. Agents Chemother. 45, 2157–2159.
35. Rattan, A., Kalia, A., and Ahmad, N. (1998). Multidrug-resistant Mycobacterium tuberculosis: molecular perspectives. Emerg. Infect. Dis. 4, 195–209.
36. Somoskovi, A., Parsons, L.M., and Salfinger, M. (2001). The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in Mycobacterium tuberculosis. Respir. Res. 2, 164–168.
37. Herrera, L., Jimenez, S., Valverde, A., Garci, Aranda, M.A., and Saez-Nieto, J.A. (2003). 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. Int. J. Antimicrob. Agents 21, 403–408.
38. Zhang, Y. and Mitchison, D. (2003). The curious characteristics of pyrazinamide: a review. Int. J. Tuberc. Lung Dis. 7,6–21.
39. Zimhony,O.,Vilcheze,C.,and Jacobs,W.R.,Jr. (2004). Characterization of Mycobacterium smegmatis expressing the Mycobacterium tuberculosis fatty acid synthase I (fas1) gene. J. Bacteriol. 186,4051–4055.
40. Scorpio, A. and Zhang, Y. (1996). Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat. Med. 2, 662–667.
41. Louw, G. E., Warren, R. M., Donald, P. R., Murray, M. B., Bosman, M, van Helden, P. D., Young, D. B., and Victor, T. C. (2006). Frequency and implications of Pyrazinamide resistance in managing previously treated tuberculosis patients. Int.J.Tuberc.Lung Dis. In Press
42. Takayama, K. and Kilburn, J.O. (1989). Inhibition of synthesis of arabinogalactan by ethambutol in Mycobacterium smegmatis. Antimicrob. Agents Chemother. 33, 1493–1499.
43. Johnson, R., Jordaan, A.M., Pretorius, L., Engelke, E., van der, S.G., Kewley, C., Bosman, M., van Helden, P.D., Warren, R., and Victor, T.C. (2006). Ethambutol resistance testing by mutation detection. Int. J. Tuberc. Lung Dis. 10, 68–73.
44. Brzostek, A., Sajduda, A., Sliwinski, T., Augustynowicz-Kopec, E., Jaworski, A., Zwolska, Z., and Dziadek, J. (2004). Molecular characterisation of streptomycin-resistant Mycobacterium tuberculosis strains isolated in Poland. Int. J. Tuberc. Lung Dis. 8, 1032–1035.
45. van Rie, A., Warren, R., Mshanga, I., Jordaan, A.M., van der Spuy, G.D., Richardson, M., Simpson, J., Gie, R.P., Enarson, D.A., Beyers, N., van Helden, P.D., and Victor, T.C. (2001). Analysis for a limited number of gene codons can predict drug resistance of Mycobacterium tuberculosis in a high-incidence community. J. Clin. Microbiol. 39, 636–641.
46. World Health Organization. Guidelines for drug susceptibility testing for second-line anti-tuberculosis drugs for DOTS-plus. 2001.
47. Cheng, A.F., Yew, W.W., Chan, E.W., Chin, M.L., Hui, M.M., and Chan, R.C. (2004). Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fluoroquinolone-resistant Mycobacterium tuberculosis clinical isolates. Antimicrob. Agents Chemother. 48, 596–601.
48. Cynamon, M.H. and Sklaney, M. (2003). Gatifloxacin and ethionamide as the foundation for therapy of tuberculosis. Antimicrob. Agents Chemother. 47, 2442–2444.
49. Ginsburg, A.S., Grosset, J.H., and Bishai, W.R. (2003). Fluoroquinolones, tuberculosis, and resistance. Lancet Infect. Dis. 3, 432–442.
50. Suzuki, Y., Katsukawa, C., Tamaru, A., Abe, C., Makino, M., Mizuguchi, Y., and Taniguchi, H. (1998). Detection of kanamycin-resistant Mycobacterium tuberculosis by identifying mutations in the 16S rRNA gene. J. Clin. Microbiol. 36, 1220–1225.
51. Morlock, G.P., Metchock, B., Sikes, D., Crawford, J.T., and Cooksey, R.C. (2003). ethA, inhA, and katG loci of ethionamide-resistant clinical Mycobacterium tuberculosis isolates. Antimicrob. Agents Chemother. 47, 3799–3805.
52. Feng, Z. and Barletta, R.G. (2003). Roles of Mycobacterium smegmatis D-Alanine:D-Alanine Ligase and D-Alanine Racemase in the Mechanisms of Action of and Resistance to the peptidoglycan D-Cycloserine. Antimicrob. Agents Chemother. 47, 283–291.
53. Taniguchi, H., Chang, B., Abe, C., Nikaido, Y., Mizuguchi, Y., and Yoshida, S.I. (1997). Molecular analysis of kanamycin and viomycin resistance in Mycobacterium smegmatis by use of the conjugation system. J. Bacteriol. 179, 4795–4801.
54. Victor, T.C., van Helden, P.D., and Warren, R. (2002). Prediction of drug resistance in M. tuberculosis: molecular mechanisms, tools, and applications. IUBMB. Life 53, 231–237.
55. Kim, B.J., Lee, K.H., Yun, Y.J., Park, E.M., Park, Y.G., Bai, G.H., Cha, C.Y., and Kook, Y.H. (2004). Simultaneous identification of rifampin-resistant Mycobacterium tuberculosis and nontuberculous mycobacteria by polymerase chain reaction-single strand conformation polymorphism and sequence analysis of the RNA polymerase gene (rpoB). J. Microbiol. Methods 58, 111–118.
56. Mohamed, A.M., Bastola, D.R., Morlock, G.P., Cooksey, R.C., and Hinrichs, S.H. (2004). Temparature mediated heteroduplex analysis for detection of pncA mutations

associated with pyrazinamide resistance and differentiation between Mycobacterium tuberculosis and Mycobacterium bovis by denaturing high- performance liquid chromatography. J. Clin. Microbiol. 42, 1016–1023.

57. Newton, C.R., Graham, A., Heptinstall, L.E., Powell, S.J., Summers, C., Kalsheker, N., Smith, J.C., and Markham, A.F. (1989). Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Res. 17, 2503–2516.
58. Piatek, A.S., Telenti, A., Murray, M.R., el Hajj, H., Jacobs, W.R., Jr., Kramer, F.R., and Alland, D. (2000). Genotypic analysis of Mycobacterium tuberculosis in two distinct populations using molecular beacons: implications for rapid susceptibility testing. Antimicrob.. Agents Chemother. 44, 103–110.