Chapter - 3

Review of Literature
Tuberculosis (TB) is one of the leading causes of death in the world today. The World Health Organization (WHO) estimates that *Mycobacterium tuberculosis* caused active disease in 9.15 million people across the globe, killing 1.6 million of them. More people carry the bacillus today—one-third of the world’s population—than at any other period in history. (WHO 2007) Despite the availability of drugs to cure tuberculosis (TB) since the 1940s, TB remains an important cause of death from an infectious agent, second only to the human immunodeficiency virus, or HIV (WHO 2004f).

*Mycobacterium tuberculosis*, the pathogen responsible for TB, uses diverse strategies to survive in a variety of host lesions and to evade immune surveillance. Their cell walls contain mycolic, acid-rich, long-chain glycolipids and phospholipoglycans (mycocides) that protect mycobacteria from cell lysosomal attack and also retain red basic fuchsin dye after acid rinsing (acid-fast stain).

![Figure -3.1 Discoverer of TB Bacilli- Robert Koch](image)

In 1882, Robert Koch (figure 3.1) identified *Mycobacterium tuberculosis* as the causative agent of TB, but since his discovery the global TB epidemic seems unabated; this year it is anticipated that there will be about 9.8 million new cases, more than in any other year in history (Dye. et al, 2010). This situation highlights the relative shortcomings of the current treatment strategies for TB and the limited effectiveness of public health systems, particularly in resource-poor countries where
the main TB burden lies. The ease with which TB infection spreads (for example, by inhalation of a few droplet nuclei 2–5 μm in diameter containing as few as 1–3 bacilli (Riley., 1957), has helped to sustain this scourge at current levels. Inhaled infectious droplets lodge in the alveoli, and bacilli are taken up there by macrophages, beginning a series of events that result in either the containment of infection or the progression to active disease (Frieden et al. 2003).

Following uptake by macrophages, *M. tuberculosis* replicates slowly but continuously and spreads through the lymphatic system to hilar lymph nodes. In most infected people, cell-mediated immunity, associated with a positive tuberculin test, develops two to eight weeks after infection. Activated T lymphocytes and macrophages form granuloma, which limit the further replication and spread of bacilli. Unless a later defect occurs in cell-mediated immunity, the infection remains contained within the granuloma. In spite of half a century of anti-TB chemotherapy, one-third of the world’s population asymptotically still harbor a dormant or latent form of *M. tuberculosis* with a lifelong risk of disease reactivation (Figure 3.2).

![Figure 3.2 Transmissions and reactivation of TB bacilli](image)

**Figure 3.2 Transmissions and reactivation of TB bacilli**

Reactivation of latent TB, even after decades of subclinical persistence, is a high risk factor for disease development particularly in immunocompromised individuals such as those co-infected with human immunodeficiency virus (HIV), on an anti-tumour necrosis factor therapy or with diabetes (Barry, et al. 2009).
• It has been estimated that for every one percent annual risk of tuberculosis infection, there are about
  – 50 new pulmonary sputum smear positive (NSP) cases
  – 50 new pulmonary sputum smear negative (NSN) cases
  – 25 re-treatment pulmonary cases
  – 10 extra-pulmonary cases
  – Totaling to 135 cases ……………per 100,000 population per year.

• This means that, with an ARTI of 1.6% for West Zone (including Gujarat), there will be
  – 80 new smear positive cases,
  – 80 new smear-negative cases,
  – 40 re-treatment cases, and
  – 16 extra-pulmonary cases,
  – Totaling to 216 cases ……………per 100,000 populations per year.

**Epidemiology**

The World Health Organization (WHO) has estimated that 2 billion people have latent TB and that globally, in 2009, the disease killed 1.7 million people. (WHO. 2010) Approximately 3.9 million cases were sputum-smear positive, the most infectious form of the disease (Corbett et al, 2003; Dye et al, 1999; WHO 2005). The African region has the highest estimated incidence rate (345 per 100,000 populations annually), but the most populous countries of Asia harbor the largest number of cases: Bangladesh, China, India, Indonesia, and Pakistan together account for half the new cases arising each year. In terms of the total estimated number of new TB cases arising annually, about 80 percent of new cases occur in the top-ranking 22 countries.

The world’s two most populous countries, India and China, account for more than 50% of the world’s MDR-TB cases and as such these countries are encountering a high and increasing TB disease burden, which is also expected because these two
countries are two most populated countries of the world and a major part of their population cannot afford expensive treatment.

Annually around 1 million people die from TB with MDR-TB and XDR-TB (extensive drug-resistant-TB) claiming most of the lives (WHO, 2010). The sheer size of their TB case populations results in the highest estimated numbers of MDR-TB cases (about 100,000 each) emerging annually from these two countries. Moreover, the emergence of XDR strains of *M. tuberculosis* (5.4% of MDR-TB cases are found to be XDR-TB (WHO, 2010)) is challenging TB treatment programmes in several other countries and even raises the possibility of a return to a situation akin to the pre-antibiotic TB era.

**HIV and TB:**

The increasing rate of human immunodeficiency virus (HIV) infection in many countries has had an impact on tuberculosis (TB) epidemiology. While TB prevalence has remained stable, TB incidence continues to rise, especially in countries most severely affected by the HIV epidemic as well as those facing political turmoil, migration, poverty and unemployment and where intravenous drug abuse is rampant. HIV is the most important known risk factor that promotes progression to active TB in people with *Mycobacterium tuberculosis* infection (TB/HIV A Clinical Manual 2004). The lifetime risk of tuberculosis in immunocompetent persons is 5% to 10%, but in HIV positive individuals, there is a 5% to 15% annual risk of developing active TB disease (Swaminathan *et al* 2000). WHO estimated 9.2 million new cases of TB globally in 2006 (139 per 100,000); of whom 7, 09,000 (7.7%) were HIV positive (World Health Organization 2008). India, China, Indonesia, South Africa and Nigeria rank first to fifth in terms of incident TB cases. TB accounts for about one in four of the deaths that occur among HIV-positive people (WHO. 2010). Of the 9.4 million TB cases in 2009, 11–13% were HIV positive with approximately 80% of these co-infections confined to the African region (WHO.2010). In India, there were 2.5 million people living with HIV and AIDS (PLWHA) at the end of 2007 while the incidence of TB was approximately 1.8 million cases per year (WHO Release 2007, RNTCP 2008). In a survey carried out among new tuberculosis patients by the Revised National TB control Program (RNTCP) in 2007, HIV sero-prevalence varied widely and ranged from 1% to
13.8% across the 15 districts (Central TB Division, unpublished observations). Currently, it is not clear what role the HIV epidemic has played in the TB situation in India.

HIV and TB form a lethal combination, each speeding the other's progress. HIV weakens the immune system. Someone who is HIV-positive and infected with TB bacilli is many times more likely to become sick with TB than someone infected with TB bacilli that is HIV-negative. TB is a leading cause of death among people who are HIV-positive. In Africa, HIV is the single most important factor contributing to the increase in the incidence of TB since 1990 (WHO 2010). Persons with AIDS are 20-40 times more likely than immune-competent persons to develop active TB. (WHO 2010) Correspondingly, TB is the leading cause of mortality among persons infected with HIV (CDC 2009).

Although HIV/AIDS and MDR-TB linkage is uncommon (Glynn et al., 2002, Kenyon et al., 1999), it has been observed in some outbreaks of MDR-TB (Gordin et al., 1996). Strains associated with HIV/AIDS form larger clusters (Mistry et al., 2002) and represent actively transmitted strains. There is a likely explanation for the association between HIV/AIDS and MDR-TB: if in AIDS patients *Mycobacterium tuberculosis* continues replicating in the continuation phase, it will result in exposure of bacilli to rifampicin alone because isoniazid has a shorter half-life, as there are no other supporting drugs (Dye et al., 2002). The studies from Mumbai (Mistry et al., 2002, Almeida et al., 2003) also showed the presence of dominant clusters as well as unique strains. A single strain creating an MDR-TB epidemic, (e.g., the Beijing strain) is unlikely in this scenario because of an internal competition between the resistant strains.

**Diagnosis**

After many years of scientific quiescence, tuberculosis (TB) research has undergone a renaissance. Although truly major advances that would revolutionize TB diagnosis, treatment, and prevention have not been realized, we are beginning to see the innovations that have been prompted by the recognition of the economic potential of the market for new diagnostic tests and treatments for TB and considerably increased public and private funding.
Figure 3.3 10-year diagnostic pipeline. Timeline of diagnostic tests currently endorsed by the WHO, as well as those that are under development and review by Richard O’Brien (Foundation for Innovative New Diagnostics).

Till now we don’t know the very clear picture of drug resistance from many part of the world (WHO, 2010) and diagnosis is the key factor in the spreading of drug resistant TB. So in order to control the spread of drug resistant TB, diagnosis should be accurate and on time and to accomplish this purpose diagnostic centre and other health care services should be easily accessible by the patients. The diagnosis can be done by conventional methods like, a) absolute concentration method, b) the resistance ratio method and c) proportion method using Lowenstein Jensen culture for testing drug sensitivity or by newer methods like radiometric methods, e.g., Benton-Dickinson method Franklin lakes, BACTEC-460 etc. Also, the methods like mycobacteria indicator tube (MGIT) system, restriction fragment length
polymorphism (RFLP) patterns to categorize different isolates of MTB for better understanding of molecular epidemiology of TB, use of ligase chain reaction (LCR), luciferase reporter assay, FASTPlaque TB-RIL, a rapid bacteriophage based test, polymerase chain reaction (PCR), the Line Probe Assay (LiPA) are also being used for better analysis of MDR-TB and XDR-TB (Sharma et al. 2004, 2006). Determination of resistance to a given drug is performed as an *in-vitro* assay in the laboratory, a process called drug susceptibility testing (DST). Where resources are limited, the WHO recommends a hierarchy of DST that should include at least R and H the two most efficacious drugs that define MDR-TB (Rich et al.2006). For more than 40 years, DST in the developing countries has relied on conventional *indirect* susceptibility methods on Lowenstein-Jensen (LJ) solid medium (Canetti et al.1963). Indirect testing involves primary isolation of pure colonies of *Mycobacterium tuberculosis*, which are then used as inoculum for DST. In contrast, *direct* DST involves inoculation of processed smear positive samples rather than pure MTB colonies. Results of direct testing are much more rapid and help to triage MDR from non-MDR-TB patients promptly.

**Conventional Susceptibility Tests**

Three conventional techniques - the proportion method, the absolute concentration and resistance ratio have been standardized, and are widely used in the developing countries (Aziz et al.2003).

**Proportion method**

With this method, an equal quantity of a standardized inoculum of *M. tuberculosis* is seeded on a drug-free and drug-containing medium. The drug free medium is seeded with an inoculum that is 100 times diluted compared with that seeded on the drug-containing medium. Distinct, countable colony-forming units (CFU) should be present on the drug-free medium. On the drug-containing medium, only pre-existing resistant mutants are expected to grow. Although the proportion of pre-existing mutants based on a mutation rate of 1 in $10^7-10^9$ would be much lower, for ease of interpretation, it is theoretically assumed to be 1%, and this has been determined to predict therapeutic outcome (Canetti et al.1969). Assuming that 1% of the inoculum on the drug medium are resistant mutants, only these mutants will grow, and by dividing the number of CFU on drug medium by those on drug free medium it is possible to deduce that the
isolate is susceptible (≤ 1%) or resistant (>1%). Thus to interpret as susceptible, the number of CFU on the drug medium must not exceed those on drug free medium. This is the principle underlying the proportional method of DST in MTB (Canetti et al.1963, 1969). The proportion method can be performed on LJ or Middlebrook agar medium (Makinen et al.2006). The LJ medium is recommended by the WHO and the IUATLD for developing countries as it is cheap, easy to read, has low contamination rates and DST results are highly reproducible (WHO 1997). With the PM, estimation of the inoculum size from the colony- forming units (CFU) counts is easy. However, a single CFU could arise from a clump of bacilli rather than from an individual cell, resulting in an inaccurate calculation of the proportion of resistant mutants and thus false results. The LIJM is the DST method commonly used in India and in many other developing countries.

*Critical concentrations of drugs:* The critical concentration (CC) is defined as the concentration that Inhibits *in-vitro* growth of most MTB cells within the population of wild type strains without appreciably affecting the growth of pre-existing resistant mutants (Canetti et al.1969).

**The absolute concentration method**

An inoculum of *M. tuberculosis* is added to LJ or 7H10/7H11 agar containing several sequential dilutions of each drug. Resistance is indicated by the lowest concentration of the drug that Inhibits growth, *i.e.* fewer than 20 colonies by the end of 4 weeks (IUATLD 1998).

**The resistance ratio method**

The resistance ratio (RR) is the ratio of the minimum Inhibitory concentration (MIC) for the patients’ strain to the MIC of the drug- susceptible reference strain, H37Rv, both tested in the same experiment (Heifets L. 2000). After 4 weeks of incubation, growth on any slope is defined as the presence of 20 or more colonies, and MIC is defined as the lowest drug concentration where the number of colonies is less than 20. A resistance ratio of 2 or less indicates sensitive strain, and a resistance ratio of 8 or more indicates resistant strains (Canetti et al.1963). The RR method is the most expensive of the three conventional methods (Heifets L. 2000).

Conventional tests have been time tested to offer very reproducible DST results and have been considered as the gold standard tests for TB susceptibility testing. However,
when performed on solid medium - typical of RLSs, the DST process is very slow (2-3 months), necessitating the need for more rapid assays.

New Rapid Susceptibility Tests

The new rapid susceptibility tests in the literature included **Solid media culture-based techniques** such as Nitrate Reductase Assay (NRA) (Angeby et al. 2002), E test (AB Bio Disk Solna, Sweden) (Wanger et al. 1994, Joloba et al. 2000), and Phage-based susceptibility tests (Biotec Laboratories Ltd., Ipswich, UK, (Albert et al. 2004, Simboli et al. 2005); **In-house liquid media culture-based tests** such as the microscopic observation drug susceptibility (MODS) assay (Moore et al. 2006), Alamar blue (da Silva et al. 2006), the MTT test (3-(4,5- dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) (Mengatto et al. 2006), and resazurin assays (Montoro et al. 2005); **commercial liquid media culture-based tests** such as the BACTEC 460 radiometric system (Becton Dickinson, Sparks, Maryland), Mycobacterium Growth Indicator Tube- MGIT (Becton Dickinson, Sparks, Maryland), and MB BacT/Alert system (bioMe’rieux, Marcy l’Etoile, France) (Woods GL 2000, Piersimoni et al. 2006); and **Molecular tests** (Line Probe Assays) such as the INNO-LiPA Rif. TB Assay (Innogenetics, Ghent, Belgium) (Jureen et al. 2004) and the Genotype® MTBDR and its newer version - the Genotype® MTBDRplus (Hain Life sciences, Nehren, Germany) (HainLifescience. 2009).

Solid Media Culture-Based Tests

**Nitrate reductase assay (NRA).** *Mycobacteria tuberculosis* has nitro- reductase enzymes that catabolically reduce nitrate (NO3) to nitrite (NO2), in the reaction pathway:

\[
\text{KNO}_3 + 2e^- + 2H \rightarrow \text{NO}_2 + 2H_2O
\]

In 1879, Griess, a German chemist working at the University of Marburg, described the diazotization reaction, which now forms the basis for the Griess test for the detection of nitrite (Griess et al. 1879). By incorporating 1mg/mL potassium nitrate (KNO3) in the medium, the reduction of nitrate to nitrite can be detected using the Griess reagent. When Griess reagent is added on the 7th-21st day of incubation, the nitrite in the medium causes a pink-purplish color. In the presence of R or H at the
critical concentrations, the appearance of a pink-purple colour represents resistance to the drug (Angeby et al. 2002). Susceptible strains do not grow, as they are inhibited by the antibiotic thus producing a non-coloured reaction. As the NRA uses the detection of nitrate reduction as an indicator of growth, DST results can be obtained faster than by waiting for visual detection of colonies.

Progress had been made in the use of the NRA for indirect DST in *M. tuberculosis* showing sensitivity and specificity of 92-100% (Martin et al. 2008). The NRA test is technically easy to set and read, and gives a clear cut answer on susceptibility. When NRA is used for indirect DST, the bio safety and cost is almost similar to the LJPM since it needs a minor modification to perform the test. However, data on the performance of the NRA in RLSs in India was limited (Singh et al. 2008). It was therefore essential to further evaluate the NRA test procedures before it gets considered for routine MDR-TB diagnosis in RLS.

**E test susceptibility testing:** This method uses plastic strips that contain exponential gradients of antibiotics for susceptibility testing of mycobacteria (AB BIODISK, Solna, Sweden). The antibiotic diffuses into the medium and thereby inhibiting growth of susceptible strains. The minimum inhibitory concentration (MIC) is read and the isolate interpreted as resistant or susceptible. Initial studies of the E test showed high accuracy estimates of close to 100% when compared with the conventional agar proportion and BACTEC radiometric tests (Wanger et al. 1994, Joloba et al. 2000). However, it is now known that the diffused antibiotics degrade fast amidst the slowly growing mycobacteria resulting in a blurred cut off point for MIC reading. The other disadvantage of the E test is the need for a heavy inoculum *i.e. #3* MacFarland (MF) equivalent, which may not be achievable with direct DST on sputum sediments, but which also poses a major risk of aerosol generation and inhalation by the staff in the safety level 2 laboratories of developing countries. The E test antibiotic strips are also very expensive (up to USD 30 per strip), which may not be affordable in RLSs. With these issues, the E test was not found to be suitable for further evaluation in this research programme.

**Phage-based susceptibility tests.** Phage assays rely on the ability of live and thus resistant *M. tuberculosis* pre-incubated with the test drug to support the growth of an
infecting mycobacteriophage - a virus that infects mycobacteria (Simboli et al. 2005, McNerney R. 2001). Both the commercially available FastPlaque TB assay and the in-house versions have been mainly studied for the detection of rifampicin resistance of either M. tuberculosis isolates or directly on clinical specimens with good results and rapid time to results of 2-3 days (Albert et al. 2004, Simboli et al. 2005). An evaluation of this assay in Uganda also showed high sensitivity and specificity (Traore et al. 2007). However, the phage assay can be technically complex, labour intensive, and can have high failure rates with inability to have interpretable results (Joloba M personal communication). This test was excluded from further evaluation.

**In-House Liquid Media Culture-Based Tests**

*Microscopic-observation drug-susceptibility (MODS) assay* The MODS assay is a broth-based technique for the detection of tuberculosis and multidrug-resistant tuberculosis, indirectly or directly from sputum. The test relies on three principles (Moore et al.2006): first, Mycobacterium tuberculosis grows faster in liquid medium than in solid medium; second, characteristic cord formation occurs and these cords can be visualized microscopically in liquid medium at an early stage; and third, incorporation of drugs permits rapid and direct drug-susceptibility testing concomitantly with the detection of bacterial growth. Resistant strains are detected due to the ability of M. tuberculosis to grow with characteristic cord-like structures detected with an inverted microscope. Visualization of cord-like structures in liquid medium containing the tested drug indicates resistance. Recently, the protocol for the MODS assay has been updated to include a well with Para-Nitrobenzoic Acid (PNB) to help identify MTB from atypical mycobacteria. PNB inhibits growth of MTB complex but not atypical mycobacteria (Rastogi et al. 1989).

Studies on MODS have shown sensitivities and specificities ranging from 86-100% for rifampicin and isoniazid resistance (Moore et al.2006, Park et al.2002). The MODS assay requires minimal training, is easy to set and results are rapid (7-14 days). However, microscopic observation of the cords may be subjective. Being a liquid culture-based test performed on tissue culture plates, concerns have been raised over the bio safety of staff working with this test. However, the main bio safety concern is at the point of sputum processing and inoculation after which the plate is supposedly sealed and never re-opened even at microscopic examination.
**Alamar blue, Resazurin and MTT assays.** These tests are referred to as colorimetric assays since they involve oxidation-reduction reactions with a colour change (Martin et al. 2007). They all use liquid medium on 96-well micro titre plates although tube assays have been reported (WoldeMeskel et al. 2005). Supplemented 7H9 broth containing the test drug is inoculated with mycobacteria and incubated for 7 days at 37°C. After addition of Alamar blue or resazurin reagents to wells and if there is bacterial growth, the blue oxidized reagent is reduced to a pink dye visible with the naked eye or with a colorimeter. A change of colour from blue to pink in a drug-containing well indicates presence of growing resistant *M. tuberculosis* (Reis et al. 2004). For the MTT assay, detection of resistance is based on the ability of mitochondrial dehydrogenase enzymes from viable mycobacterial cells to cleave the tetrazolium rings of the pale yellow MTT, resulting in formation of violet-purple or dark blue formazan, visible with a naked eye or with a colorimeter (Montoro et al. 2005). After the 7 days incubation the yellow MTT is added to the wells, and the plate incubated for 24 hours to allow the MTT to precipitate in the cytoplasm. A lysing buffer is then added to the wells to lyse the bacterial cell and release the MTT into the medium. Development of a strong violet-purple colour in the drug-containing well indicates presence of resistant mycobacterial strains (Montoro et al. 2005).

Each of these colorimetric methods has been assessed in previous studies with reported sensitivity and specificity of 94-100%, and the results obtained within 10 days (Martin et al. 2007). These tests use micro titre plates with around 10 samples per plate, thus high throughput, which would be good for TB high-burden settings. However, after 7 days of incubation, the Alamar blue and resazurin plates are opened once while the MTT plate must be opened twice to add the detection reagents. This is not only cumbersome but also carries a serious bio safety risk to the laboratory personnel. On basis of high throughput these tests were listed for further analysis to assess their performance in India even though they but may not be optimal for safety level-2 TB laboratories.
Commercial Liquid Media Culture-Based Tests

**BACTEC 460.** The BACTEC 460 (Becton Dickinson, Sparks, Maryland) relies on radiometric detection of $^{14}\text{C}O_2$ as an indicator of bacterial growth. The Bactec vials contain Middlebrook 7H12 medium and fatty acid substrates labeled with $^{14}\text{C}$. Growing mycobacteria release $^{14}\text{C}O_2$ as a metabolic end product. The gas is removed, analyzed and the amount of radioactive $^{14}\text{C}$ is expressed as a numerical value called the Growth Index (GI). When the GI value in the control vial reaches 30 interpretation of drug tube begins on the next day as follows. Susceptible: Change in GI in the control vial > GI in drug vial; Resistant: Change in GI in control vial < GI in drug vial and Border line: GI in control vial = GI in drug vial. This test is highly sensitive and specific but it uses radioactive carbon whose half life is 5,000 years, which makes it difficult and expensive to dispose. Due to these issues, the BACTEC 460 is being phased out and has been replaced by non-radiometric systems such as the Mycobacterial Growth Indicator Tube and the MB/BactAlert system. The BACTEC 460 test was therefore not evaluated further in this study.

**Mycobacterial Growth Indicator Tube** The Mycobacterial Growth Indicator Tube (MGIT; Becton Dickinson, Sparks, Maryland, USA) is based on fluorescence detection of mycobacterial growth in a tube containing a modified Middlebrook 7H9 medium together with fluorescence quenching-based oxygen sensor (a ruthenium pentahydrate substance embedded in silicone rubber) at the bottom of the tube (Reisner et al. 1995, Rusch-Gerdes et al.1999). As the bacteria grow and consume oxygen, the indicator fluoresces under ultraviolet light, and growth in a tube with the test drug indicates resistance (Reisner et al. 1995). The MGIT system, introduced around 15 years ago, in its manual and now automated versions, is part of the new-generation of rapid tests for detection of drug resistant TB. Studies of both the manual and automated MGIT 960 system have shown very high correlation with conventional DST methods for rapid detection of resistance to the first and second-line anti-TB drugs (Johansen et al. 2004, Pfyffer et al.1997, Idigoras et al. 2000).

The automated MGIT 960 system has the advantage of high throughput (>900 samples can be tested on one instrument at ago), it is rapid (4-13days), and very
easy to interpret results. All these aspects would be suitable for the TB high-
burden settings. However, the test has been studied only as an indirect assay
mainly in the developed countries.

Thus, there is limited data on the technical performance of the test in RLS, and on
how the operational issues such as cost, contamination rates, and power failures
would impact on the DST results in the low income settings. In this study, the semi
automated MGIT system was evaluated.

The BacT/ALERT ® 3D System. The BacT/ALERT ® 3D System (bioMe´rieux,
Marcy Etoile France) is a liquid based automated assay performed in a tube with a
liquid emulsion sensor. Growing bacteria produce CO2, which reacts with the sensor,
resulting is a colour change from gray to a lighter colour, detected colorimetrically as
growth. Growth in a drug tube indicates resistance. The BacT/ALERT ® 3D System
is slightly slow compared with the MGIT 960. This test was thus excluded from
further analysis.

Molecular Assays
Molecular methods for MDR-TB detect the common mutations conferring resistance
to R and H, rather than the resistance phenotype. The commercially available line
probe assays involve DNA extraction, polymerase chain reaction (PCR), and solid
phase reverse hybridization of amplified DNA to probes covering the core region of
the target gene, immobilized on a nitrocellulose strip. These tests can be applied on
MTB isolates or on sputum smear positive sputum (De Beenhouwer et al. 1995,
Rossau et al. 1997).

The INNO-LiPA Rif TB Assay (Innogenetics, Ghent, Belgium) detects the
common mutations in only the rpoB gene for rifampicin resistance (Rossau et
al.1997). Evaluation studies of the INNO-LiPA Rif. TB Assay showed high
sensitivity and specificity (Jureen et al. 2004). Rifampicin resistance predicts MDR-
TB in over 90% of cases, and may be sufficient for MDR diagnosis. However,
isoniazid testing as well may be helpful in the design of second line drug regimens
for MDR-TB patients. The GenoType® MTBDR assay (Hain Lifesciences, Nehren,
Germany) simultaneously detects the common mutations in the rpoB and katG gene
(Hillemann et al. 2005). The GenoType® MTBDRplus, a newer version of the genotype MTBDR detects more of the common mutations in the rpoB and katG genes, and also mutations in the inhA promoter region, making it the most sensitive line probe assay for detection of resistance (Hillemann et al. 2007). Evaluation studies of these assays have reported sensitivity and specificity of 98-100% for rifampicin, and of 70-100% for isoniazid, with results in 1-3 days (Ling et al. 2008).

Most of these studies were performed in developed countries and there was limited data on the performance of the tests in developing countries (Ling et al. 2008). A major limitation of these assays in developing countries could be the expertise in molecular biology required to perform them correctly, the unidirectional work flow laboratory infrastructure and the cost of molecular assays. However, a study on the INNO-LiPA Rif. TB assay in Rwanda demonstrated that the required skills could be learnt in a matter of weeks (Quezada et al. 2007). Additionally, many of the developing countries now have facilities for basic molecular biology as used in monitoring viral load in HIV treatment. To conclude this section, apart from the intrinsic properties, the performance of diagnostic tests also depends on the prior probability of disease in the study population, and the design of the study (Irwig et al. 2002). The design of some of the studies cited above was typical of in stage 1 or 2 of test development, and was mostly conducted in the resource-rich settings (Knotterus et al. 2002). Some of the studies were done on very diverse or intentionally biased study populations, casting uncertainty on the wider applicability of the results, particularly in the RLSs (Martin et al. 2007, 2008, Ling et al. 2008,). Furthermore, recent MDR-TB diagnostic research has focused on direct susceptibility testing. However, data on the listed tests when used as direct assays was very limited. The WHO in July 2010 recommended the use of colorimetric tests and the NRA for TB susceptibility testing in RLSs, but the available data to support the recommendation of for example the NRA in RLSs was admittedly limited (WHO. 2010). In this thesis we provide more recent data and experience with these assays in a typical RLS.
Treatment

Currently, the only means of immunizing against TB is with the live attenuated vaccine BCG, although other vaccines are under development (Fruth and Young 2004; Goonetilleke and others 2003; Horwitz and others 2000; Letvin, Bloom, and Hoffman 2001; Reed and others 2003; Young and Stewart 2002). Randomized controlled trials and case-control studies have shown consistently high protective efficacy of BCG against serious forms of disease in children 73 percent for meningitis and 77 percent for miliary TB but highly variable and often very low efficacy against pulmonary TB in adults (Bourdin Trunz, Fine, and Dye, forthcoming; Fine 2001; Rieder 2003). Thus, even with the high coverage now achieved, BCG is unlikely to have any substantial effect on transmission. Recently used First line and Second line drugs listed below in Table 3.1 (Davies, 2006).

Table 3.1 Drugs recently used for the treatment of tuberculosis

<table>
<thead>
<tr>
<th>Table of drugs used for the treatment of tuberculosis.</th>
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<tr>
<td><strong>First line drugs</strong></td>
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<td>Essential</td>
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<td>Isoniazid</td>
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<td>Rifampicin</td>
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<td>Streptomycin</td>
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<td>Rifabutin</td>
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<td>Rifapentine</td>
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In recent years, the TB epidemic has been further fuelled by the emergence of multi- and extensively-drug-resistant (MDR-TB and XDR-TB) strains and dwindling treatment options that are decades old. Rifampicin is a member of the last novel class of antibiotics introduced in 1963 for first-line treatment of tuberculosis. Drugs in this class are part of a 6-month regimen that is ineffective against multidrug-resistant and extensively drug resistant tuberculosis, and have unfavorable drug interactions with
many antiretroviral drugs. Except for the addition of the fluoroquinolones as second-line therapy, nothing was added until 2000 (Adhvaryu et al. 2011) as shown in Figure 3.3

**Figure 3.4: Time-line for TB drug development**

At present, MDR-TB is treated by a combination of eight to ten drugs with therapies lasting up to 18–24 months; only four of these drugs were actually developed to treat TB (Gandhi et al. 2010). Such suboptimal therapy leads to almost 30% of MDR-TB patients experiencing treatment failure (Mitnick et al. 2003). XDR-TB has a higher mortality rate than MDR-TB as the treatment options for XDR-TB are very limited as XDR-TB bacilli are resistant not only to isoniazid and rifampicin, but also to fluoroquinolones and injectable such as aminoglycosides.

Designing drugs for MDR-TB and XDR-TB is extremely difficult as in the beginning the tubercle bacilli may remain in dormant state for a long time and grow slowly. Moreover, the perturbations in the genes may make it unsusceptible to many drugs which may be able to penetrate the cell wall, disrupt the eradication mechanism in macrophages and lie there creating a second permeability barrier (Scheindlin et al. 2006)
The principles for the treatment of both MDR-TB and XDR-TB are same. Extensive chemotherapy is carried out for about 2 years (WHO. 2008). The anti-TB drugs should be consumed according to the prescribed daily dosage by WHO to prevent drug resistance in MTB. Some big challenges are also associated with the dual AIDS-TB treatment regimen i.e. drug–drug interaction, drug–disease interactions, immune reconstitution inflammatory syndrome (IRIS), shared drug toxicities, and high pill burdens (Pepper 2009).

**VACCINE**

Even the most experienced healthcare experts in the developed world are frequently stunned to learn that TB infects one-third of the world's population. While most of these infections are latent, between 5 and 10 percent of infected individuals develop active, contagious disease and suffer significant debility or death. The TB bacterium, *M. tuberculosis*, is extremely slow-growing compared to other bacteria. As a result, *M. tuberculosis* is inaccessible or resistant to many of the powerful antibiotics that are effective against other bacterial infections. Those few drugs that are effective are used in courses of combination treatment that can take more than six months. In recent years, multi-drug-resistant TB strains (MDR-TB) have arisen that are resistant to standard treatment and require extensive and costly treatment with new cocktails of potent, toxic drugs.

Even if novel, highly potent, and rapid-acting drugs were available, they still would not reduce the vast reservoir of TB-infected patients. The path to reducing the epidemic levels of TB infection, particularly in the poorest countries of the world, is through a TB vaccine. Such a vaccine provided it is safe, effective, and affordable would have profound impact worldwide on death and morbidity from this widespread disease.

Fortunately, we now have a better understanding of how the organism causes disease and how the host immune system responds to *M. tuberculosis* infection. The sequencing of the *M. tuberculosis* genome has led to real advances in vaccine development and accelerated the identification of novel antigens that are protective in animal models. As a result, there are nearly a dozen vaccine candidates in the pipeline, with several promising candidates in late stages of pre-clinical development and three in human clinical trials.
Renewed public interest in, and funding for, TB vaccine development, combined with a revolution in vaccine development technology, has brought forth several new approaches to TB vaccine design over the past decade. The leading candidates fall into two categories: live mycobacterial vaccines (genetically engineered TB or BCG) and subunit vaccines (genetically engineered TB proteins combined with immunostimulants or viral vector vaccines using genes for TB antigens expressed by viral carriers).

The pipeline includes two distinct product profiles:

- Vaccines that aim to replace the existing BCG vaccine with improved duration of immunologic memory (more than 20 years) for newborns (typically live attenuated strains); and
- Vaccines targeted toward children, adolescents, and adults as a boost to the neonatal BCG vaccination (typically subunit vaccines or viral vectored vaccines).

Both are prophylactic vaccines to be given prior to TB infection.

The live attenuated vaccine candidates intended to replace BCG include genetically modified BCG vaccines (live attenuated M. bovis) and live attenuated M. tuberculosis. These vaccines are aimed at immunologically naïve recipients. Given widespread use of BCG, a replacement vaccine would need to demonstrate superior efficacy to BCG to be seriously considered.

Development of effective subunit vaccines has progressed considerably with the sequencing of the M. tuberculosis genome. There are now several subunit vaccines under development, including fusion protein vaccines and the use of viral vectors for key antigens. These vaccines, which would be used as booster vaccines, aim to boost immunity in infants or young adults already primed by earlier vaccination with BCG as an infant. However, since not all individuals will have been vaccinated as infants, booster vaccines should be able to stimulate effective primary responses as well.

Experts in tuberculosis generally agree that ultimately what is needed is a combined neo-natal and adult vaccine regimen—or “prime-boost” strategy—if TB immunity is to continue through the adult years. While not evaluated as part of this study, a therapeutic vaccine to treat individuals already infected with TB is also needed before a broad-spectrum solution will be in hand.

Several promising candidates are in early stages of development, with three in human
clinical trials. Oxford University’s viral vector vaccine candidate (MVA85A) is currently in Phase II trials and, if Phase II testing is successful, Oxford expects to enter Phase III trials in 2008. GlaxoSmithKline and Statens Serum Institute/Intercell have subunit vaccines under development that are expected to enter Phase III testing in 2010. Several other BCG-replacement and booster vaccines are scheduled to begin Phase I trials within 12 months. Given the range of products in development and anticipated development timelines, we expect that at least one new vaccine will successfully complete Phase III testing and be licensed by 2013-2015.

Drug resistance
Development of resistance to anti-tuberculosis drugs was recognized shortly after the initial introduction of chemotherapy for the treatment of tuberculosis. The large majority of patients treated with streptomycin in the first Medical Research Council randomized clinical trial in the 1940s acquired resistance to that drug (MRCI.1948). The spread of drug-resistant strains was soon recognized, and a survey of clinics in England in the 1950s found that >5% of patients with tuberculosis who had no history of previous treatment had strains resistant to at least 1 of the 3 major drugs in use at that time (Fox et al.1957). It is known today that at least 3 effective drugs used in combination are needed to treat tuberculosis while preventing development of drug resistance (Zhang et al.2009). However, despite the introduction of combination regimens throughout the world many years ago, the presence of drug resistance has been progressively documented from an ever wider geographical area (Wright et al.2009). Recent estimates by the World Health Organization (WHO) suggest that nearly half million cases of multidrug-resistant tuberculosis emerged globally in 2008 (WHO. 2010).

Drug resistance may be transmitted (also called primary resistance) or acquired.

- Primary resistance, which occurs when the infecting strain is already resistant to ≥1 antituberculosis drug at the time of its first encounter with the subject, is an indicator of transmission in the community.
- Acquired resistance, defined when the patient's bacterial population acquires resistance during treatment consequent to exposure to inadequate therapy, is an indication of poor patient adherence to treatment, caregiver errors in

**Causes of drug-resistant tuberculosis**

Drug-resistant TB has microbial, clinical, and programmatic causes. From a microbiological perspective, the resistance is caused by a genetic mutation that makes a drug ineffective against the mutant bacilli. An inadequate or poorly administered treatment regimen allows drug-resistant mutants to become the dominant strain in a patient infected with TB. Table 3.2 summarizes the common causes of inadequate treatment. However it should be stressed that MDR-TB is a man-made phenomenon poor treatment, poor drugs and poor adherence lead to the development of MDR-TB.

**Table 3.2 Causes of inadequate treatment**

<table>
<thead>
<tr>
<th>Providers/Programmes: Inadequate regimens</th>
<th>Drugs: Inadequate supply/quality</th>
<th>Patients: Inadequate drug intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Absence of guidelines inappropriate guidelines</td>
<td>-Non-availability of certain drugs (stock-outs or delivery disruptions)</td>
<td>-Poor adherence (or poor DOT)</td>
</tr>
<tr>
<td>-Non-compliance with guidelines</td>
<td>-Poor quality</td>
<td>-Lack of information</td>
</tr>
<tr>
<td>-Inadequate training of health staff</td>
<td>-Poor storage conditions</td>
<td>-Non-availability of free drugs</td>
</tr>
<tr>
<td>-No monitoring of treatment</td>
<td>-Wrong dosages or combination</td>
<td>-Adverse drug reactions</td>
</tr>
<tr>
<td>-Poorly organized or funded TB control programmes</td>
<td></td>
<td>-Social and economic barriers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Malabsorption</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Substance abuse disorders</td>
</tr>
</tbody>
</table>

**MDR**

Multidrug-resistant TB (MDR-TB) is caused by bacteria that are resistant to at least isoniazid and rifampicin, the most effective anti-TB drugs. MDR-TB results from either primary infection with resistant bacteria or may develop in the course of a patient’s treatment. Extensively drug-resistant TB (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 fluoroquinolones 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 (WHO, 2010).

Mortality rates for patients with XDR-TB are similar to those of patients from the preantibiotic era (Approximately 1 in 13 *M tuberculosis* isolates currently shows a form of drug resistance.) (CDC, 2009). The emergence of resistance to drugs used to treat tuberculosis (TB), and particularly multidrug-resistant TB (MDR-TB), has become a significant public health problem in a number of countries and an obstacle to effective TB control. In India, the available information from the several drug resistance surveillance studies conducted in the past suggest that the rate of MDR-TB is relatively low in India. However this translates into a large absolute number of cases and as yet the management of patients with MDR-TB is inadequate. Specific measures are being taken within the Revised National Tuberculosis Control Programme (RNTCP) to address the MDR-TB problem through appropriate management of patients and strategies to prevent the propagation and dissemination of MDR-TB.

Traditionally, DOTS-Plus refers to DOTS programmes that add components for MDR-TB diagnosis, management and treatment. These guidelines promote full integration of DOTS and DOTS-Plus activities under the RNTCP, so that patients with MDR-TB are both correctly identified and properly managed under the recommendations set out in this document.

Finally, the guideline introduces new standards for registering, monitoring and reporting outcomes of multidrug-resistant TB cases. This uniform information management system will allow systematic, consistent data collection and analysis which will facilitate appropriate supervision and monitoring of the DOTS Plus activities and will play an important role in shaping future policies and recommendations.

It is well known that resistance levels are higher in areas with a poorly performing DOTS programmes. Use of inadequate regimens and inappropriate directly observed treatment (DOT) leads to increase in drug resistance levels in the community. It has been acknowledged that good treatment is a pre-requisite to the prevention of emergence of resistance. RNTCP recognizes that implementation of a good quality DOTS programme is the first priority for TB control in the country. Prevention of
emergence of MDR-TB in the community is more imperative rather than its treatment. DOTS-Plus services, for management of MDR-TB, are supplementary services under the expanded framework of the DOTS package. Therefore in every DOTS implementing unit of the country, DOTS would be prioritized above DOTS-Plus with the view that DOTS reduces the emergence of MDR-TB, and therefore the need for DOTS plus over time.

Over the past few years, the basic package of DOTS for TB control has been expanded in many areas to include components that address additional challenges such as TB/HIV co-infection, multidrug-resistant TB, contributing to health system strengthening, engaging all care providers, empowering patients and communities, and enabling and promoting research. Emphasis on expanding laboratory capacity (smear microscopy first, then culture/drug sensitivity testing) and the use of quality assured drugs, are important parts of this more comprehensive approach to TB control.

The first WHO endorsed DOTS-Plus programmes began in 2000. At that time, the Green Light Committee (GLC) was established to promote access to high quality second-line drugs for appropriate use in TB control programmes. DOTS-Plus pilot projects have demonstrated the feasibility and effectiveness of MDR-TB treatment in less affluent countries. In 2002, the Global Fund to fight AIDS, TB, and Malaria (GFATM) started financing TB control programmes, including MDR-TB, thus greatly reducing the economic barrier to MDR-TB control. Since then, DOTS-Plus projects have multiplied rapidly. By the end of 2007, 67 projects in 52 countries approved by the GLC, with a cumulative total of over 30,000 MDR-TB patients, had been launched worldwide, many of them with financial support from the GFATM. Based on data and experience from these projects, practices and further scientific evidence have emerged regarding services for MDR-TB. DOTS-Plus programmes can and should strengthen the basic DOTS strategy.

Potential causes of MDR-TB and XDR-TB:

- **Factors related to treatment history:** In most of the countries which are adversely affected by drug resistant TB, public health care system is not very good and private practitioners are not used to maintain the treatment history of patient and due to this patient are subjected to be treated improperly.
- **Improper and incomplete treatment:** Mismanagement of the treatment for instance, prescribing a single drug and later on adding up off another drug to the collapsing regimen, undiagnosed pre-existing resistance to drugs, prescribing inadequate regimen, inadequate follow up of the regimen by the patient results in the reduced susceptibility of the patient to anti-TB drugs. Non-adherence to the prescribed regimen is mostly ignored by the clinicians dealing with the treatment which makes the patient susceptible to MDR-TB and XDR-TB (Sharma et al, 2006, Mitchison, 1998, Frieden et al, 1993.) The patients are reluctant to adhere to the prescribed regimen due to the adverse effects of the most of the anti-TB drugs (Table 1) (Sharma et al, 2006). Incomplete treatment and improper regimen, both poses a selection pressure on the drug resistant strains that’s why the prevalence of MDRTB is higher among the retreated patients compared to the new cases..

- **Infrastructure:** Due to the lack of well equipped laboratories and facilities for growing culture for determining the sensitivity of the Mycobacterium to the applied drugs in developing nations, the diagnosis often made inferring the treatment history of the patient (which is also not very well maintained in most of the cases) and logarithms. (Sharma et al, 2006).

- **Mutation:** Mutations at the different sites in the different genes in MTB are actually responsible for the resistance caused for the drugs. Every drug has a unique target in MTB and when there are perturbations in these target sites the drug is rendered inefficient for that specific purpose for which it has been designed. Genes responsible for drug resistance in MTB after mutations are listed in (Table 2).

- **Multi-drug transporter proteins:** Multidrug transporter proteins mediate both acquired and intrinsic resistance to anti-TB drugs. The human analogue for this protein is P-glycoprotein’s, which is expressed on the effectors cells of immune system and has been shown to be over expressed in the experimental cell lines on the infection of MTB hence the accumulation of isoniazid inside the cells is reduced (Sharma et al, 2004).

The proportion of MDR-TB among new TB cases reported globally ranges from 0% to 28.3% (Figure 3.4). Asia has reported proportions of MDR-TB among new cases exceeding 6%. While the TB case populations of China and India may have
proportions of MDR-TB lower than Eastern European and Central Asian countries, the sheer sizes of the two countries’ TB case populations result in the highest estimated numbers of MDR TB cases emerging annually in these two countries: approximately 100 000 cases each (WHO 2008).

**Figure 3.5 Distribution of proportion of MDR- TB among new cases, 1994-2009**

The proportion of MDR-TB among previously treated TB cases reported globally ranges from 0% to 61.6% (Figure 3.5). The data provided in this report confirm that the highest proportions of MDR-TB are found in countries of Eastern Europe and Central Asia. These high proportions explain in part the slow progress made in Eastern European and Central Asian countries in reaching the Millennium Development Goal target of halving TB mortality rates by 2015 compared with their levels of 1990 (WHO 2009).
Figure- 3.6 Distribution of proportion of MDR-TB among previously treated TB cases, 1994-2009

Figure- 3.7 Any resistance among previously treated TB cases, 1994-2007
Table 3.3 Scenario of TB, HIV and MDR –TB in India

<table>
<thead>
<tr>
<th>MDR-TB estimates of burden *</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>% of new TB cases with MDR-TB</td>
<td>2.3 (1.8–2.8)</td>
<td>[DRS 2005]</td>
</tr>
<tr>
<td>% of retreatment TB cases with MDR-TB</td>
<td>17 (15–20)</td>
<td>[DRS 2005]</td>
</tr>
<tr>
<td>MDR-TB cases among incident total TB cases in 2008</td>
<td>99,000 (79,000–120,000)</td>
<td></td>
</tr>
<tr>
<td>MDR-TB cases among new pulmonary TB cases notified in 2009</td>
<td>23,000 (18,000–28,000)</td>
<td></td>
</tr>
<tr>
<td>MDR-TB cases among retreated pulmonary TB cases notified in 2009</td>
<td>50,000 (43,000–57,000)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MDR-TB notified cases 2009</th>
<th>New</th>
<th>Retreatment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed cases of MDR-TB</td>
<td>1,660</td>
<td>1,660</td>
<td></td>
</tr>
<tr>
<td>MDR-TB patients started treatment</td>
<td>1,136</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of MDR-TB patients living with HIV/AIDS</th>
<th>No representative data available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds of HIV-positive TB patient having MDR-TB over odds of HIV-negative TB patient having MDR-TB</td>
<td>No representative data available</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Estimates of burden * 2009</th>
<th>Number (thousands)</th>
<th>Rate (per 100,000 pop)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality (excluding HIV/AIDS)</td>
<td>280 (160–430)</td>
<td>23 (14–36)</td>
</tr>
<tr>
<td>Prevalence (incl HIV/AIDS)</td>
<td>3,000 (1,300–5,000)</td>
<td>249 (105–419)</td>
</tr>
<tr>
<td>Incidence (incl HIV/AIDS)</td>
<td>2,000 (1,600–2,400)</td>
<td>168 (137–202)</td>
</tr>
<tr>
<td>Case detection, all forms (%)</td>
<td>67 (56–83)</td>
<td></td>
</tr>
</tbody>
</table>

* Ranges represent uncertainty intervals (WHO 2011)
Resistance to second-line anti-TB drugs, including XDR-TB

By March 2011, a total of 69 countries reported to have identified at least one case of XDR-TB (Figure 3.7). In 2008, 963 cases of XDR-TB were reported to WHO globally from 33 countries compared with 772 cases from 28 countries in 2007. Many XDR-TB cases are believed to be never diagnosed due to weaknesses in laboratory capacity to test for resistance to second line drugs.

A total of 46 countries, distributed across the six WHO regions (Table 3.4) have reported continuous surveillance or representative survey data on second-line drug resistance among MDR-TB cases.

![Map 1: Global distribution of countries reporting at least one XDR-TB case by March 2011](image)

**Figure 3.8 Global distributions of countries reporting at least one XDR-TB case by March 2011**

**XDR-TB Findings:** • 58 countries reported at least one case of XDR-TB as of March 2010 • Representative data from 46 countries • 5.4% of MDR-TB cases have XDR-TB
Table 3.4 Number of countries reporting data on resistance to second-line anti-TB drugs, WHO region

<table>
<thead>
<tr>
<th>WHO region</th>
<th>No. of countries reporting second-line anti-TB drug resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African (46)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Americas (35)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Eastern Mediterranean (21)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>European (53)</td>
<td>31 (58)</td>
</tr>
<tr>
<td>South-East Asia (11)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Western Pacific (27)</td>
<td>6 (22)</td>
</tr>
<tr>
<td><strong>Total (193)</strong></td>
<td><strong>46 (24)</strong></td>
</tr>
</tbody>
</table>

Table 3.5 The estimated number of MDR-TB cases (primary and acquired) by WHO region.

<table>
<thead>
<tr>
<th>WHO region</th>
<th>Estimated number of MDR-TB cases (primary and acquired) in 2008 (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African</td>
<td>69 000 (53 000–110 000)</td>
</tr>
<tr>
<td>Americas</td>
<td>8 200 (7 300–9 300)</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>24 000 (11 000–81 000)</td>
</tr>
<tr>
<td>European</td>
<td>81 000 (73 000–90 000)</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>130 000 (110 000–170 000)</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>120 000 (100 000–140 000)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>440 000 (390 000–510 000)</strong></td>
</tr>
</tbody>
</table>
Need of the hour

The urge of the present scenario of MDR-TB and XDR-TB is to develop reliable and rapid detection method of MDR and XDR strains of MTB. Containment of the spread of multidrug-resistant and extensively drug-resistant tuberculosis will be extremely difficult without treatment regimens that are shorter, safer, more effective, and less expensive than those presently available. New drugs with novel mechanisms of action are needed for effective management of multidrug-resistant and extensively drug-resistant tuberculosis (Ma 2010).

One study documented the emergence of new forms of totally drug resistant bacilli among patients with multidrug-resistant disease. The isolation of totally drug-resistant strains from multidrug-resistant tuberculosis patients from different regions is alarming, and indicates possible dissemination of such strains in Asian countries (Velayati 2009). Present clinical trials are testing new combinations of drugs for their safety and efficacy in treatment durations of 6 months for drug-susceptible tuberculosis, as well as new drugs in optimized regimens for multidrug-resistant tuberculosis (Lienhardt 2010).

Synthesized compound

Benzothiozinones and dinitrobenzamide

These agents were found to be highly active against resistant and sensitive strains of M. tuberculosis, including extensively drug-resistant and multidrug-resistant strains. Both BTZ043 (benzothiozinone and dinitrobenzamide) drugs have the same target, i.e., heterodimeric decaprenylphosphoryl-D-ribose 2-epimerase, encoded by the dprE1(Rv3790) and dprE2(Rv3791) genes (Christophe 2009), thereby blocking arabinogalactan and lipoarabinomannan synthesis in the mycobacterial cell wall and growth of M. tuberculosis (Makarov 2009).

To monitor for potential development of benzothiazinone resistance, a total of 240 sensitive and multidrug-resistant M. tuberculosis clinical isolates from four European hospitals were surveyed for the presence of mutations in the dprE1 gene and for benzothiazinone susceptibility. All 240 strains were susceptible, thus establishing a baseline prior to the introduction of BTZ043 in clinical trials. The EPFL School of
Life Sciences is the current developer of this drug that has now entered Phase I clinical investigation in humans (Pasca 2010).

**Newly synthesized 1,2,4-triazoles with benzothiazoles**

The standard therapy for TB includes isoniazid, targeting both the NADH-dependent enoyl reductase (InhA) and the 3-oxoacyl ACP syntheses (KasA) and rifampicin, a well characterized inhibitor of the DNA-dependent RNA-polymerase. There are two basic approaches to develop a new drug for TB: (i) synthesis of analogues, modifications or derivatives of existing compounds for shortening and improving TB treatment and, (ii) searching novel structures, that the TB organism has never been presented with before, for the treatment of multidrug resistant TB (Patel N. (2010).

To pursue this goal, our research efforts are directed to find new chemical classes of Antitubercular active agent with different mode of action. The azole antitubercular may be regarded as a new class providing truly effective drugs which are reported to inhibit the bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanism (Babaoglu 2003, Shiradkar 2007a, 2007b, 2007c, Dabak 2003,). Triazoles in particular, substituted-1,2,4-triazole are among various heterocycles that have received the most attention during last two decades as potential antimicrobial agents, antifungal, antitubercular, anti-HIV, anti-inflammatory, CNS stimulants, sedatives, antianxiety (Joshi 2008, Zampieri 2009, Elkholy 2006, Kaplancikli 2008, Ulusoy 2001, Ozdemir 2007, Muhi-Eldeen 1991, Kucukguzel 2008, Banfi 2001).

Benzothiazole moiety has already been reported for its antimicrobial and antitubercular activity (Vicini 2003, Gaspora 1997) along with antitumor, anti-inflammatory, analgesic, anticonvulsant, diuretic, antimalarial, antidiabetic (Stanton 2008, Stefania 2008, Palagiano 1996, Jimonet 1999, Singh 2006, Burger 1968, Moreno-Diaz 2008, Chunying 2007). After extensive literature search, it was observed that, till date enough effort have not been made to combine this two moieties as a single molecular scaffold and to identify new candidates that may be value in designing new, potent, selective and less toxic Antitubercular and antimicrobial agents. In view of this data, we reported the synthesis of new 1,2,4-triazoles incorporated with benzothiazole and pyridine which possessed wide variety of biological activity encouraging Antitubercular activity against *M. tuberculosis* H37 Rv and antimicrobial activity.
Herbal compounds

Moreover, people are trying to find out the treatment for drug resistant TB on the basis of Indian traditional knowledge of medicinal plants and people have identified such medicinal plants which show significant activity against drug resistant strains of MTB (Gupta et al, 2010)

Due to the increasing problem of antibiotic/drug resistance, WHO recommended exploring herbs or plants as alternative remedy for various bacterial infections. The antimicrobial properties of plants have been investigated by a number of researchers worldwide and the results are very promising (Cowan, 1999).

**Garlic** (*Allium sativum*) is natural plant being used as a food as well as folk medicine for centuries in all over the world (Rivlin, 2001). In 1996, Reuter *et al.* described garlic a plant with various biological properties like antimicrobial, anti-cancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic, and anticardiovascular effects (Reuter *et al.*, 1996). Different garlic extracts demonstrated activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, *clostridium*, *Helicobacter pylori* (Cellin *et al.*, 1996) and even acid-fast bacilli (AFB) such as MTB (Uchida *et al.*, 1975). Allicin is thiosulfinate compound of garlic reported for its antibacterial activity. Allicin is proved to be anti-bacterial as it inhibits RNA synthesis (Feldberg *et al.*, 1988). According to Ayurvedic and Greek systems of medicine garlic is one of the established remedies for tuberculosis. In 1946 Rao *et al.* firstly described the *in-vitro* garlic activity against *Mycobacterium tuberculosis* (Rao *et al.*, 1946). A few studies have also been proving antimycobacterial activity of garlic against different species of mycobacteria (Gupta *et al.*, 1955; Abbruzzese *et al.*, 1987; Jain, 1998; Gupta *et al.*, 1999; Bolton *et al.*, 1982; Deshpande *et al.*, 1993; Delaha and Garagusi, 1985).

The aim of the present study was to evaluate antimycobacterial activity of Ethanolic garlic extract (EGE) against clinical isolates of MDR *Mycobacterium tuberculosis* by using a recently discovered, most sensitive and rapid 7H9 middle brook broth dilution technique, which is a fluorescence based technique for detection of MTB.

**Turmeric** is a spice which is obtained from rhizomes of plant *Curcuma longa*. Components of turmeric are named curcuminoids, which include mainly curcumin
(diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin (Chainani 2003) (Figure 3.8).

![Figure 3.9 Chemical structures of curcuminoids](image)

Turmeric consists of 3-5% curcuminoids. Curcumin is the most important fraction which is responsible for the biological activities of turmeric. The melting point of curcumin, C\(_2\)H\(_{20}\)O\(_6\), is 184°C. It is soluble in ethanol and acetone, but insoluble in water (Joe 2004).

![Figure 3.10 Turmeric plant](image)  ![Figure 3.11 Garlic plant](image)

Curcumin exists in solution as keto-enol tautomers (Payton 2007). Because of its biological activities, a large number of studies have been presented on curcumin. According to these studies, curcumin exhibits antiinflammatory (Chainani 2003) antioxidant (Kunchandy 1990, Masuda 1993, Unnikrishnan 1995, Cohly 1998) anticarcinogenic (Frank 2003) antiviral (Suai 1993) antimicrobial activity (Mahady 2002, Han 2005). Beside these, curcumin has a variety of potentially therapeutic
properties, such as antineoplastic, antiapoptotic, antiangiogenic, cytotoxic, immunomodulatory, (Strimpakos 2008) and antithrombotic, wound healing, antidiabetogenic, antistressor and antilithogenic actions (Chainani 2003). Biological assessment of turmeric and curcumin have been discussed against standard bacterial and mycobacterial strains such as *E.coli, S.aureus, E.feacalis, P.aeuroginosa, M.smegmatis, M.simiae, M.kansasii, M. terrae, M.szulgai* and the fungi *Candida albicans*(Cikrikci 2008).