CHAPTER 1
1 INTRODUCTION TO TUBERCULOSIS

1.1. HISTORY AND DEVELOPMENT OF MYCOBACTERIUM TUBERCULOSIS

1.1.1. INFECTION AND TRANSMISSION

*Mycobacterium tuberculosis* (Mtb), the main causative organism of tuberculosis (TB), is a successful pathogen that overcomes the numerous challenges presented by the immune system of the host. TB remains one of the world's greatest causes of mortality and morbidity, with approximately 8 million new infections and 2 million deaths per year (Dye *et al.*, 1999). More adults die due to TB every year than AIDS and malaria together (Corbett *et al.*, 2003). It is difficult to kill Mtb for a number of reasons such as its slow growth, dormancy, complex cell envelope, intracellular pathogenesis and genetic homogeneity. TB is a contagious disease, like common cold, which spreads the infection through air. Only people who are sick with TB in their lungs are infectious, when infectious people cough, sneeze, talk or spit, they propel TB germs, known as bacilli, into the air. A person needs only to inhale a small number of these to be infected. The fifth annual report on global tuberculosis control of the World Health Organization found that there were an estimated 8.4 million new cases up from 8.0 million in 1997. So far, 50 million people have been infected with drug-resistant Mtb strains, and very few drugs have been developed in the past 40 years (Chopra *et al.*, 2003). Left untreated, each person with active TB disease will infect on average between 10 to 15 people every year. But people infected with TB bacilli will not
necessarily become sick with the disease. The immune system "walls off" the TB bacilli which, protected by a thick waxy coat, can lie dormant for years and when the immune system is weakened, the chances of becoming sick are greater.

Someone in the world is newly infected with TB bacilli every second, in an overall data analysis one-third of the world's population is currently infected with the TB bacillus, 5-10% of people who are infected with TB bacilli (but who are not infected with HIV) become sick or infectious at some time during their life and people with HIV and TB infection are much more likely to develop TB.

1.1.2. Global and regional incidence

The World Health Organization (WHO) estimates that the largest number of new TB cases in 2005 occurred in the South-East Asia Region, which accounted for 34% of incident cases globally. However, the estimated incidence rate in sub-Saharan Africa is nearly twice that of the South-East Asia Region, at nearly 350 cases per 1,00,000 population. It is estimated that 1.6 million deaths resulted from TB in 2005. Both the highest number of deaths and the highest mortality per capita are in the Africa Region. The TB epidemic in Africa grew rapidly during the 1990s, but this growth has been slowing each year, and incidence rates now appear to have stabilized or begun to fall. In 2005, estimated per capita TB incidence was stable or falling in all six WHO regions. However, the slow decline in incidence rates per capita is offset by population growth. Consequently, the number of new cases arising each year is still increasing globally and in the WHO regions of Africa, the Eastern Mediterranean and South-East Asia. Table 1.1 below shows the estimated TB incidence (the number of new cases arising each year) and mortality in each of the WHO regions

1.1.3. HIV and TB

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 incidence of TB since 1990.

Table 1.1 WHO Fact sheet N°104, March 2007

Estimated TB Incidence, Prevalence and Mortality, 2005

<table>
<thead>
<tr>
<th>WHO region</th>
<th>Incidence a (thousands)</th>
<th>Prevalence a (thousands)</th>
<th>TB Mortality a (thousands)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All forms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smear-positive b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>2 529 (29)</td>
<td>1 088 (147)</td>
<td>3 773 (511)</td>
</tr>
<tr>
<td>The Americas</td>
<td>352 (4)</td>
<td>157 (18)</td>
<td>448 (50)</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>565 (6)</td>
<td>253 (47)</td>
<td>881 (163)</td>
</tr>
<tr>
<td>Europe</td>
<td>445 (5)</td>
<td>199 (23)</td>
<td>525 (60)</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>2 993 (34)</td>
<td>1 339 (81)</td>
<td>4 809 (290)</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>1 927 (22)</td>
<td>866 (49)</td>
<td>3 616 (206)</td>
</tr>
<tr>
<td>Global</td>
<td>8 811 (100)</td>
<td>3 902 (60)</td>
<td>14 052 (217)</td>
</tr>
</tbody>
</table>

a Incidence - new cases arising in given period; prevalence - the number of cases which exist in the population at a given point in time.
b Smear-positive cases are those confirmed by smear microscopy, and are the most infectious cases. Pop indicates population.

WHO and its international partners have formed the TB/HIV Working Group, which develops global policy on the control of HIV-related TB and advises on how those fighting against TB and HIV can work together to tackle this lethal combination. The interim policy on collaborative TB/HIV activities describes steps to create mechanisms of collaboration between TB and HIV/AIDS programmes, to reduce the burden of TB among people and reducing the burden of HIV among TB patients.

1.1.4. Drug-resistant TB

Until 50 years ago, there were no medicines to cure TB. Now, strains that are resistant to a single drug have been documented in every country.
surveyed; what is more, strains of TB resistant to all major anti-TB drugs have emerged. Drug-resistant TB is caused by inconsistent or partial treatment, when patients do not take all their medicines regularly for the required period because they start to feel better, because doctors and health workers prescribe the wrong treatment regimens, or because the drug supply is unreliable. A particularly dangerous form of drug-resistant TB is multidrug-resistant TB (MDR-TB), which is defined as the disease caused by TB bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs. Rates of MDR-TB are high in some countries, especially in the former Soviet Union, and threaten TB control efforts, while drug-resistant TB is generally treatable, it requires extensive chemotherapy (up to two years of treatment) with second-line anti-TB drugs which are more costly than first-line drugs, and which produce adverse drug reactions that are more severe, though manageable. Quality-assured second-line anti-TB drugs are available at reduced prices for projects approved by the Green Light Committee. The emergence of extensively drug-resistant (XDR) TB, particularly in settings where many TB patients are also infected with HIV, poses a serious threat to TB control, and confirms the urgent need to strengthen basic TB control and to apply the new WHO guidelines for the programmatic management of drug-resistant TB.

1.1.5. The Stop TB Strategy, the Global Plan to Stop TB, 2006–2015 and targets for TB control

In 2006, WHO launched the new Stop TB Strategy, the core of this strategy is DOTS, the TB control approach launched by WHO in 1995. Since its launch, more than 22 million patients have been treated under DOTS-based services and the new six-point strategy builds on this success, while recognizing the key challenges of TB/HIV and MDR-TB. It also responds to access, equity and quality constraints, and adopts evidence-based innovations in engaging with private health-care providers, empowering affected people and communities and helping to strengthen health systems and promote research.
The six components of the Stop TB Strategy are:

- **Pursuing high-quality DOTS expansion and enhancement.**

  Making high-quality services widely available and accessible to all those who need them, including the poorest and most vulnerable, requires DOTS expansion to even the remotest areas. In 2004, 183 countries (including all 22 of the high-burden countries which account for 80% of the world’s TB cases) were implementing DOTS in at least part of the country.

- **Addressing TB/HIV, MDR-TB and other challenges.** Addressing TB/HIV, MDR-TB and other challenges requires much greater action and input than DOTS implementation and is essential to achieving the targets set for 2015, including the United Nations Millennium Development Goal relating to TB.

- **Contributing to health system strengthening.** National TB control programmes must contribute to overall strategies to advance financing, planning, management, information and supply systems and innovative service delivery scale-up.

- **Engaging all care providers.** TB patients seek care from a wide array of public, private, corporate and voluntary health-care providers. To be able to reach all patients and ensure that they receive high-quality care, all types of health-care providers are to be engaged.

- **Empowering people with TB, and communities.** Community TB care projects have shown how people and communities can undertake some essential TB control tasks. These networks can mobilize civil societies and also ensure political support and long-term sustainability for TB control programmes.

- **Enabling and promoting research.** While current tools can control
TB, improved practices and elimination will depend on new diagnostics, drugs and vaccines.

The strategy is to be implemented over the next 10 years as described in The Global Plan to Stop TB, 2006–2015. The Global Plan is a comprehensive assessment of the action and resources needed to implement the Stop TB Strategy and to achieve the following targets:

- Millennium Development Goal (MDG) 6, Target 8: Halt and begin to reverse the incidence of TB by 2015.

- Targets linked to the MDGs and endorsed by the Stop TB Partnership:
  - by 2005: detect at least 70% of new sputum smear-positive TB cases and cure at least 85% of these cases.
  - by 2015: reduce TB prevalence and death rates by 50% relative to 1990.
  - by 2050: eliminate TB as a public health problem (1 case per million population) and progress towards targets.

In 2005, an estimated 60% of new smear-positive cases were treated under DOTS – just short of the 70% target, treatment success in the 2004 DOTS cohort of 2.1 million patients was 84% on average, close to the 85% target. However, cure rates in the African and European regions were only 74%, the 2007 WHO report Global TB Control concluded that both the 2005 targets were met by the Western Pacific Region, and by 26 individual countries (including 3 of the 22 high-burden countries: China, the Philippines and Viet Nam). The global TB incidence rate had probably peaked in 2005, and if the Stop TB Strategy is implemented as set out in the Global Plan, the resulting improvements in TB control should halve prevalence and death rates in all regions except Africa and Eastern Europe by 2015.
1.2. Impact of Mtb genome sequence on identification of new drug targets

The complete genome sequence of *M. tuberculosis* H37Rv provides an opportunity for a more focused and planned approach towards the identification of new drug targets. Genome sequence helps in compilation of all the potential gene products encoded by a particular organism, identification of functions (enzymes and pathways) that are missing or unique in a particular organism and finally identifying the genes that are common to all (or most) prokaryotes and eukaryotes. An important advantage of this analysis is the possibility of identifying a novel target that is present in many bacteria and subsequently designing a drug that could be active against a wide range of bacteria.
In addition, availability of human genome sequence can help in eliminating the potential drug targets that have close human homologues. Thus, the possibilities of using complete genome sequences for target identification are virtually unlimited (Chitta Suresh Kumar et al., 2005).

1.2.1. Novel drug targets in \( \text{Mtb} \)

\( \text{Mtb} \) is surrounded by a lipid-rich outer capsule that protects it from the toxic radicals and hydrophobic enzymes produced as defense by host macrophages (Kolattukudy et al., 1997). The peptidoglycan layer of the cell wall serves as a base for the lipid-rich capsule. Peptidoglycan or murein is the polymeric mesh of the bacterial cell wall, which plays a critical role in protecting the bacteria against osmotic lysis. Cell wall biosynthesis can be separated into two phases—the six intracellular enzymatic steps and the three steps that occur outside of the plasma membrane. Of all the metabolic pathways the peptidoglycan pathway is a rich source of crucial targets for designing anti tuberculosis drugs and hence peptidoglycan biosynthesis pathway has been well characterized to develop a broad spectrum of antituberculosis drugs (Sharmila et al., 2005). Amongst the cytoplasmic steps involved in the biosynthesis of peptidoglycan, ligases play a major role to act as potential drug targets. One of the major ligase is D-alA-D-ala ligase (\( \text{Mtb-ddla, EC: 6.3.2.4} \)), which shows no human homologue. It is of special interest because it plays a major role in the synthesis of peptidoglycan the prime molecule which forms the backbone of \( \text{Mtb} \) cell wall. The \( \text{Mtb-ddla} \) transfers the phosphate from ATP to D-alanine on the first step of catalysis, in the second step the resulting acyl phosphate is attacked by a second D-alanine to produce a dd dipeptide following phosphate elimination (Fig 1.3).

The \( \text{Mtb-ddla} \) is considered to play a key role in D-alanine biosynthesis, which again interlinks with peptidoglycan metabolism (Fig.1.2), the metabolic pathways have been retrieved from KEGG (Kyoto's Encyclopedia of Genes and Genomes) an online pathway database (Kanehisa et al., 2002). Metabolic pathway identification numbers of the host \( H.\ sapiens \) and the pathogen (\( M.\ tuberculosis \)) were extracted from the KEGG.
database. Pathways which do not appear in the host but present in the pathogen according to KEGG database annotation have been identified as pathways unique to *M. tuberculosis* as compared to the host *H. sapiens*. Enzymes in these unique pathways were identified from the KEGG database. The corresponding protein sequences were retrieved from the KEGG database and thus based on the metabolic activity cycloserine (CS) a

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**Fig. 1.2.** Mtb-ddla (EC: 6.3.2.4) involved in D-alanine metabolism and linked to peptidoglycan metabolism.

**Fig. 1.3.** Enzymatic activity of Mtb-ddla.
structural analogue of D-alanine has been marketed clinically, despite of its excellent antimicobacterial activity it shows a series of side effects especially CNS toxicity which has limited its use. The currently used anti mycobacterial drugs are isoniazid (INH) and ethambutol (EMB). Isoniazid is known to inhibit mycolic acid synthesis (Zhang et al., 1992), whereas ethambutol inhibits the polymerization step of arabinan biosynthesis of arabinogalactan (Mikusova et al., 1995). Based on the underlying importance of the enzyme Mtb-ddla has been opted as a potential target for development of novel inhibitors. These targets were checked against the orlist of targets under investigation at the TB Structural Genomics Consortium (Celia et al., 2003). The two targets were also subjected to a cluster of orthologous groups (COGS) search to identify homologues in other pathogens. The results are shown as COG IDs in Table 1.2.

Table1. 2. Targets from unique pathways which do not have human homologue.

<table>
<thead>
<tr>
<th>KEGG ID</th>
<th>Gene Description</th>
<th>Swissprot/TrEMBL</th>
<th>Accession no</th>
<th>COG ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtu00550</td>
<td>dAlA d-Alanine-d-DL</td>
<td>DDL MYCTU</td>
<td>Rv2981c</td>
<td>COG1181</td>
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<tr>
<td>mtu00473</td>
<td>dAlA d-Alanine-d-DL</td>
<td>DDL MYCTU</td>
<td>Rv2981c</td>
<td>COG1181</td>
</tr>
</tbody>
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