1 Introduction and Epidemiology of *Mycobacterium tuberculosis* Complex in Humans

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History of Tuberculosis

Tuberculosis (TB) is arguably one of the most devastating diseases that have afflicted mankind from time immemorial. Known by many different names throughout history, such as phthisis, scrofula, consumption, King's Evil, lupus vulgaris, the white plague and 'captain of all these men of death', the scourge remains a significant public health concern. Perhaps the earliest evidence of TB comes from skeletal remains from burial sites from the latter part of the last Stone Age. Both macroscopic as well as microscopic evidence of TB, using modern scientific methods, has been found from excavations of mummified bodies from tombs from ancient Egypt dating as far back as 2400 BC (Allison *et al*., 1961; Nerlich *et al*., 1997; Zink *et al*., 2003). Drawings, pottery and statues of ancient Egypt that date up to 3000 BC have shown physical deformities that appear to show typical characteristics of TB of the spine (Vasiliadis *et al*., 2009; Dyer, 2010).

The first available writings about 'phthisis', meaning 'wasting away' in Greek, by Hippocrates (~460–370 BC) in his *Of Epidemics* dates as far back as 400 BC. Hippocrates, who is largely thought to be the father of modern medicine, believed phthisis was caused by growths in the lung, which he referred to as *tubercular*. He described phthisis as the most widespread disease of the era and provided detailed descriptions of the disease that included fevers, sweats, cough and wasting which closely resemble those of TB. The devastating nature of the disease even led Hippocrates to advise other physicians to avoid visiting 'consumption' patients with advanced disease because they would inevitably die and destroy the reputation of the attending physician. As pulmonary phthisis was commonly seen among close family members, Hippocrates and others widely considered the disease to be hereditary, a notion that persisted over a century. Aretæus, a Greek physician monk, described 'consumption' as 'a disease with a poor prognosis that was characterized by a chronic discharge of opaque, whitish yellow fluid from the lungs' (Dyer, 2010, p. 31). He associated people with a pale, slender and weak body type to be highly likely to develop TB. Another Greek physician, Clarissimus Galen (130–200 AD) downplayed the prevailing consideration of TB as a hereditary disease and instead came up with another theory that suggested transmission from person to person as

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another way by which TB could be spread. This alternate proposition ushered in the possibility, even at this very early stage, of an infectious nature of the disease that would ultimately be proved to be right. Later Girolamo Franscatoro (1478–1553), an Italian physician, suggested that phthisis could be transmitted by invisible particles which he called *seminara*, and that the disease was a result of a lung ulcer. Franscatoro was also the proponent of the use of the term *phthisis* to be restricted to the description of only pulmonary consumption instead of its common use that referred to all cases of 'wasting'. The development of techniques for performing postmortems by Andreas Vesalius (1514–1564) and his colleagues in the 16th century further advanced knowledge of TB by introducing a way in which specific symptoms could be associated with the cause of death.

The precise pathological and anatomical descriptions of the disease only began to appear in the 17th century when in 1679 a Dutch physician, Franciscus de la Boë (Sylvius), identified the 'tubercle' as a consistent characteristic change in the lungs and other areas of 'consumptive' patients. One of Sylvius' students, Thomas Willis (1621–1675), related the localized lesions in the lungs and other organs to the general wasting away of the body. Another of his students, Richard Morton (1637–1698), described the three stages of phthisis: initial inflammation, formation of tubercles, and progression to ulcers and fully fledged consumption disease. Together, Willis and Morton described a form of TB that affected lymph nodes in the neck, which they called scrofula. In 1702, Mange went on to describe the pathological features of miliary TB.

In 1720, an English physician known as Benjamin Marten described the single-celled organisms (contagious microscopic animalcula) and speculated that TB might be caused by 'wonderfully minute living creatures' which could enter the body and generate lesions and symptoms of phthisis. However, it is thought that most of his work was not taken seriously because it was not published, only appearing among daily newsprints among other non-scientific material (Doetsch, 1978). The first experimental evidence that consumption could be transmitted from humans to cattle and from cattle to rabbits was demonstrated in 1865 by Jean-Antoine Villemin, a French military surgeon. The definitive cause of TB being the tubercle bacilli was only conclusively demonstrated by the German bacteriologist Hermann Heinrich Robert Koch in 1882 when he isolated and cultured bacilli from crushed tubercles. He made his findings public at the Physiological Society of Berlin on 24 March 1882, and later in an article entitled *Die Ätiologie der Tuberculose*. Three years later, Paul Erhlich discovered the acid-fastness of the TB bacillus (Burke, 1955; Allen and Hinkes, 1982). In 1890, Koch presented findings of a material he had isolated from the tubercle bacilli. He called this tuberculin and wrote that it could 'render harmless the pathogenic bacteria that are found in a living body and do this without disadvantage to the body' (Koch, 1890). Koch even inoculated himself with the tuberculin from which he developed what he termed an unusually violent attack and fever, and also made him wonder whether the test could be used as a diagnostic test for TB (Koch, 1891). The reaction to tuberculin observation was soon picked up and used to develop a skin test that begun to be used widely as a diagnostic tool in cattle. The tuberculin test was subsequently used to assess exposure of humans to the tubercle bacilli and has remained the main screening test for TB exposure to the present day. Koch's work in unravelling the causative agent of TB was recognized with the Nobel Prize in Medicine or Physiology in 1905.

Mycobacterium tuberculosis, the organism that causes the majority of TB cases in humans, belongs to a closely related cluster of species called the *M. tuberculosis* (Mtb) complex (MTBC). This complex includes *M. bovis* (Karlson and Lessel, 1970), which primarily causes bovine TB in cattle, deer and elk, but also causes TB in humans (albeit to a lesser extent), as do *M. africanum* (Castets *et al*., 1968) and *M. canettii* (van Soolingen *et al*., 1997). Other members of the complex such as *M. microti* (Wells and Oxon, 1937), host-adapted *M. caprae (*Aranaz *et al*., 1999), *M. pinnipedii* (Cousins *et al*., 2003) and the newly described member of the Mtb complex, *M. mungi* (Alexander *et al*., 2010), have been found infecting goats, seals and banded mongooses, respectively, suggesting that if one were to look hard

enough among other social mammals, other host-adapted members of the complex could be identified (Marcel Behr, 2014, personal communication to L.E. Via). Other MTBC species would most likely be found infecting social herbivores and omnivores, as the life history of the organism requires a reasonable density of hosts for successful transmission. Recent genomic analysis of *M. canettii* strains, which have a much larger genome and colony morphology distinct from most other MTBC, has suggested that the species may be more closely related to the ancestral tubercle bacilli than the MTBC (Supply *et al*., 2013). The natural reservoir for this species, if it is not humans, is currently unknown.

Consistent documentation of TB remained unavailable until around the 17th century when TB fatalities had reached high proportions in Europe and became the major cause of death by the 20th century. Tuberculosis, which was largely considered to be a disease of the poor, had by this time become established and even afflicted royalty. Over the years it had affected many famous personalities including St Francis of Assisi, Charlotte Brontë, John Keats, George Orwell, Eleanor Roosevelt and Vivian Leigh (Moorman, 1940; Zink *et al*., 2005; Ducati *et al*., 2006).

Pathogenesis of TB and Routes of Infection

The pathogenesis of Mtb was tragically illustrated when 250 infants were mistakenly 'vaccinated' with virulent bacilli rather than the intended *M. bovis* BCG vaccine stock in Lübeck, Germany, in 1930 (Luca and Mihaescu, 2013). Twenty-nine per cent of the infants died within the first year, but another 135 showed signs of infection yet recovered unaided by existing antibiotic therapy. In the early streptomycin clinical trials of adults with pulmonary TB, roughly 50% showed improvement when assigned to bed rest alone (Fox *et al*., 1999). Once exposed to Mtb, those who do not develop primary symptomatic disease are estimated to have a 10% lifetime risk of developing clinical disease (Corbett *et al*., 2003). Tuberculosis in humans is mainly transmitted via the inhalation of infectious droplet nuclei produced by an infectious host while coughing, sneezing or talking. The lungs are the most common site of infection although TB lesions can be found in any part of the body. Other methods of transmission include inoculation and ingestion (Walker, 1910). Transmission by infection was mainly noted among butchers when bovine tuberculous material gained access to the body via small cuts and wounds. Transmission by ingestion, also fairly common at one time for bovine TB, is thought to be fairly uncommon now because most of the milk that is consumed now is pasteurized. Though rare, there have also been cases of transplacental transmission of TB (Lee *et al*., 1998; Chen and Shih, 2004; Abramowsky *et al*., 2012).

Tuberculosis infection typically begins when tubercle bacilli aerosolized by someone with infectious TB are inhaled by a susceptible host. The droplet nuclei carrying the bacilli are often small enough to be inspired to the terminal alveoli where the bacteria are engulfed by professional macrophages and may be killed. If some bacilli survive this initial innate immune response, they start replicating in the macrophage and can migrate to nearby epithelial cells (Urdahl *et al*., 2011). The bacilli can also be disseminated by macrophages to the local lymph nodes using the lymphatic system, and to other parts of the body via the bloodstream, where they can infect other cells. The inflammatory response triggered by this process results in the migration and accumulation of additional immune cells such as neutrophils and lymphocytes to the primary infection site, eventually forming the initial granulomatous lesion or Ghon focus (Gonzalez-Juarrero *et al*., 2001; Doherty and Andersen, 2005). If the immune system fails to contain the infection, bacilli in the granuloma multiply and cause the granuloma to increase in size and cellularity, which leads to necrosis, local disease spread and in some cases cavity formation in the lungs. If the bacilli are spread though the blood or lymphatic system, miliary TB may ensue. The inflammatory processes that ensue produce the typical symptoms that are seen in active TB patients, such as weakness, fever, weight loss, night sweat, chest pain, dyspnoea, cough and haemoptysis.

If the immune system manages to contain the infection, the granulomas may shrink and calcify, trapping the bacilli inside, where they can persist in a dormant, non-replicative state for a long time constituting an asymptomatic or latent TB infection. Immune competent individuals latently infected by TB have a 10% lifetime risk of developing clinical disease (Corbett *et al*., 2003). The persisting bacilli contained in the Ghon focus and other initial lesions have been hypothesized to start multiplying again due to changing host conditions including advancing age, waning of the immune system, malnutrition, alcoholism, diabetes, immunization with BCG (Stead, 1967) and human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), resulting in clinical TB disease. The premise that bacilli in granulomas are solely responsible for disease reactivation has been countered by necropsy studies that have found viable and infective bacilli in unaffected areas of the lung tissues (Feldman and Baggenstoss, 1938; Bishai, 2000) and in adipose tissues surrounding several organs (Neyrolles *et al*., 2006). More recent non-human primate studies have presented an even more complex picture, which suggests the presence of different types of lesions that vary from liquefied cavities to non-necrotic hypoxic lesions, with and without any viable bacilli with heterogeneous response to anti-TB treatment (Barry *et al*., 2009).

Early Intervention in TB Management

The milestone reached by the unequivocal demonstration by Robert Koch in 1882 that *M. tuberculosis* was the causative agent of TB did not immediately lead to significant improvement in its treatment. Early interventions were advanced by Leopold Auenbrugger (1722–1809), who associated the variety of sounds produced by tapping the chest with different symptoms of TB. Observations from this technique were later refined and used to develop a technique called percussion, which is still used today. Further breakthroughs were achieved via the discovery of X-rays by Wilhelm Conrad von Röntgen (1845–1923) in 1885, which was improved upon by Thomas Edison such that, by the 1920s, the technique proved helpful in the diagnosis and assessment of TB (Daniel, 2006).

One of the practices of treating TB that persisted for a long time was bleeding patients. Another related method was blood cupping that involved drawing blood from TB lesions with a premise that the bleeding would draw the infection from the lesions. When these practices declined, the use of various ointments, including administration of solutions such as iodine in the 1840s, was popularized until the use of cod-liver oil gained favour (Johnson, 1933).

The manner in which TB was treated changed fundamentally when a Siberian botany student, Hermann Brehmer, who had TB, was advised by his physician to seek out a healthier climate to help heal the disease. Following his physician's advice, Hermann travelled to the Himalayan mountains where he continued to work on his research and returned home free of the disease and studied medicine. After completing his medical studies in 1854, Hermann established an institution in Germany, where beds of TB patients were placed on balconies to expose them to continuous fresh air, and good nutrition was provided. This anecdotal observation led to the establishment of sanatoria throughout Europe and the USA with an emphasis on a regimen based on suitable climate, rest, good nutrition, fresh air and sunshine to treat chronic lung diseases, including TB (Kinghorn, 1921). However, sanatoria were subsequently closed down around the 1960s partly because of the slow healing process and because there was no major difference in case fatality rates among patients in sanatoria from those outside (Grzybowski and Enarson, 1978).

The next attempt at trying to cure TB was a process called collapse therapy by which TB could be cured by shrinking the lung, a technique initiated by an Italian physician Carlo Forlanini (1847–1918) in 1888. The procedure involved injecting air or nitrogen into the interpleural space, increasing the pressure until the lung collapsed. The premise was that the collapsed lung would be given a chance to rest while it repaired itself and that the process would cut off the oxygen supply to the TB bacteria, presumably killing them (Sakula, 1983).

Major advances in the management of TB were only realized following the isolation of streptomycin (the first antibiotic) by Selman Waksman in 1944; this was bactericidal against *M. tuberculosis* (Schatz *et al*., 1944). This was followed by the introduction of para-amino salicylic acid for treating TB, discovered by Jorgen Erik Lehmann (Lehmann, 1946). In 1952, Gerhard Domagk discovered yet another anti-tuberculosis drug, isoniazid (Lancaster, 1990) that would become one of the cornerstone drugs for TB treatment.

Susceptibility and Spread of TB Infection

Nearly everyone is susceptible to TB infection though the risk is higher among certain populations. The populations with a higher risk comprise individuals who have impaired immunity, and those who are constantly exposed to infectious TB patients. The latter group includes residents of high TB incidence settings, and people who either cohabit or are in close contact with infectious patients. For example, results from a recent household contact study found 6.9% of the 1206 TB contacts tested harboured *M. tuberculosis.* This study also found that most (89.2%) of the infected contacts were adults and that the majority (62.7%) of these contacts were close relatives, including 14.5% spouses (Singh *et al*., 2013). Another study detected TB infection in 64.6% of the contacts with a further 1.8% being TB culture positive. Close relatives, older age and cohabitation were also found to be associated with TB among contacts elsewhere (Sia *et al*., 2010). More recently, a review of data from studies that investigated the prevalence of latent and active TB infection and annual incidence of TB among contacts of patients with TB found that 51.5% of the contacts of TB patients in the studies from low- and middle-income settings were latently infected with TB, while 1.2% actually had active TB (Fox *et al*., 2013).

The contacts of TB patients who were found with active TB encompassed immunosuppressed individuals who lived or worked in institutionalized facilities such as hospitals, nursing homes, correctional facilities and homeless shelters. In addition, several factors have been shown to affect susceptibility to TB. Some of these factors include high bacillary load of infectious TB in the index case, proximity and length of exposure to an infectious case, co-infection with diseases or conditions that impair the immune system, malnutrition and young age (Narasimhan *et al*., 2013), abuse of alcohol and genetic factors (Davies and Grange, 2001) as well as diabetes (Kim *et al*., 1995; Alisjahbana *et al*., 2006; Jeon and Murray, 2008; Reed *et al*., 2013). Children under the age of five and those living with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are particularly prone to TB infection (Fox *et al*., 2013).

The role of genetics in susceptibility to TB has been debated for a long time (Davies *et al*., 1999). Evidence has even been demonstrated in a number of monozygotic and dizygotic twin studies (Simonds, 1957; Comstock, 1978) though it has been difficult to exclude the role of environmental factors (van der Eijk *et al*., 2007). Lately, the role of genetics in susceptibility to TB has received considerable attention, with current data suggesting an association between resistance to TB and host genetics. One reaffirmed the association of WT1 chr11 (rs2057178) genetic locus with TB susceptibility (Chimusa *et al*., 2013) and another found an association between a number of polymorphisms in the NRAMP1, VDR, HLA-DRB1 and HLA-DQB1 (Wu *et al*., 2013). Several other studies have implicated genetic polymorphisms such as the toll-like receptor 9 gene (Torres-García *et al*., 2013), HLA-A, B and DRB1 alleles (Mishra *et al*., 2013), P2X7 A1513C (rs3751143) gene polymorphism (Areeshi *et al*., 2013), genetic variations in the dicer 1, ribonuclease type III (DICER) mRNA (Song *et al*., 2013), polymorphisms in the Chr18q11.2 locus (Wang *et al*., 2013), ALOX5 (Pontillo *et al*., 2013; Shen *et al*., 2013) HSPEP1-MAFB genes (Mahasirimongkol *et al*., 2012), MRC1 polymorphism (Zhang *et al*., 2012), MCP-1 -2518 A/G polymorphism (Ben-Selma *et al*., 2011); SLC11A1 gene polymorphisms (Jin *et al*., 2009; Stagas *et al*., 2011); markers on chromosomes 15q and Xq (Bellamy *et al*., 2000), NRAMP1 and TNFA (Shaw *et al*., 1997). It has been hypothesized that resistance of a population to

TB may largely be based on the historic exposure of the population to the disease (Stead, 1992). Racial differences have been implicated in a study that administered a skin test among people in homeless shelters in which higher positive skin test results were observed among blacks compared to Caucasians. This difference has been thought to be due to the resistance to TB developed by Caucasians, particularly in Europe where TB has been endemic for a much longer period (Dubos and Dubos, 1952).

Epidemiology of TB

Today, TB constitutes one of the leading causes of morbidity and mortality worldwide, ranking only second to HIV/AIDS as the most causative agent of death. According to current data, a total of 6.1 million TB cases were reported to the World Health Organization (WHO) by national TB programmes worldwide with 5.7 million being new cases and 0.4 million cases being retreatment cases. India and China accounted for 39% of the cases, while the WHO African Region accounted for 23% of the cases in 2012. Thus, based on current estimates, between 11 and 13 million prevalent cases (equivalent to 169 cases per 100,000 population) occurred in 2012 of which about 8.6 million people (equivalent to 122 cases per 100,000 population) were incident cases, with an estimated 1.3 million fatalities. A group of 22 countries, collectively called high burden countries (HBCs) by the WHO, contributed a total of 81% of the 8.6 million global TB incident cases. The majority of the cases occurred in South-east Asia and the West Pacific Region (58%) while the African Region accounted for 27% of the total cases, and also recorded the highest rates of cases and deaths relative to population at 255 incident rates per 100,000 population (WHO, 2013). The highest contribution of cases to the global total was from India (26%) and China (12%), whereas 'South Africa and Swaziland had the highest incidence rates per capita (about one new case for every 100 people each year)' (WHO, 2013, p. 6). There was a wide variance in the TB incidence rates among countries, with the lowest being about ten cases per 100,000 population being mostly found in high-income countries and the highest rates being in low-income countries. The best estimate for the countries with the highest incidence rates was 1000 per 100,000 population per year for South Africa and Swaziland. There has been a gradual downward trend in the global incidence rates of TB from 2001, with a rate of 2% being recorded between 2011 and 2012 (WHO, 2013). Consequently, although the estimated global prevalence rate (169 cases per 100,000 population) above is still very high, it represents a 37% global decline since 1990 which, in addition to the mortality rate that has also fallen by nearly half (45%) since then, underscores the tremendous progress that has been made thus far.

With regard to the Millennium Development Goals (MDG) global 2015 targets, substantial progress has been made where a number of the set targets are within reach. These include the falling incidence rates of TB worldwide over the last decade, albeit slowly. The 45% recorded mortality rate of TB in 2015 is just 5% shy of the 50% target. In addition, the regions of the Americas and Western Pacific have already achieved the 2015 targets. Also, seven of the 22 HBCs have equally met the 2015 target for reduction of TB incidence, prevalence and mortality. However, there remain some challenges: for example, it is unlikely that the 50% reduction in TB prevalence in the community, which was at 37% in 2012, will be reached by 2015. Also, 11 of the 22 HBCs are unlikely to meet the targeted goals. The same is true with the target for MDR-TB (WHO, 2013).

Strategies of TB Control

The introduction of effective drug treatments in the mid-1940s led to tremendous declines in global TB rates until the early 1980s when the trend was reversed, in part due to the advent of HIV/AIDS. The gradual increase in the TB cases reached epidemic proportions resulting in the declaration of TB as a public health emergency by the WHO in 1993, with a call for governments worldwide to prioritize an increase in scale of TB control efforts (Raviglione, 2003). To back up this declaration,

several efforts were put in place by the WHO. The first was the launch of the recommended TB strategy control, which was later named Directly Observed Therapy Short-course (DOTS) that relied upon five elements: political commitment, case detection utilizing smear microscopy, standardized short-course chemotherapy, regular uninterrupted supply of all essential anti-TB drugs and programme supervision and evaluation. This was followed by the launch of the Stop TB Partnership in 1998 with the ambitious goal of eliminating TB as a public health problem by 2050. Other efforts to tame TB included the declaration of the MDGs by the United Nations in 2000, which committed nations to a new global partnership to reduce extreme poverty by setting out a series of time-bound targets for 2015, and advanced the cause for TB control (United Nations, 2013).

The DOTS strategy was initially developed as a public health approach to control TB in a cost-effective manner in resource-limited situations, with emphasis on prioritizing smear-positive patients. With time, however, a number of public health challenges arose, including the TB/HIV co-epidemic and the emergence of *M. tuberculosis* isolates that were resistant to at least rifampicin and isoniazid; these isolates were termed multidrug resistant (MDR). These challenges necessitated some changes in the global environment towards a more human approach to public health. This led to the redesign of disease control efforts that were more patient-centred, and directed towards universal access to care, culminating in the launch of the Stop TB Strategy in 2006 (WHO, 2006). The main goal of the Stop TB Strategy was to reduce substantially the global burden of TB by 2015 in line with the MDG and Stop TB Partnership targets and to achieve major progress in the research and development of the tools needed for TB elimination. The 2015 targets were to reduce the prevalence and mortality of TB by 50% compared to the prevailing rates in 1990. This strategy was updated to constitute the Global Plan to Stop TB 2011–2015, which provides clearer action items and guidance on what needs to be done to achieve the set goals by 2015 (WHO, 2011). The main difference between the DOTS strategy and the Stop TB Strategy was the enhancement of the concept of patient-centred care for all individuals with TB. The current Stop TB Strategy (WHO, 2013) comprises six components:

1. Pursue high-quality DOTS expansion and enhancement.
2. Address TB/HIV, MDR-TB and the needs of poor and vulnerable populations.
3. Contribute to health system strengthening based on primary health care.
4. Engage all care providers.
5. Empower people with TB and communities through partnership.
6. Enable and promote research.

TB and HIV Co-infection

Tuberculosis and HIV are responsible for the majority of the mortality observed worldwide as a result of communicable diseases. The reported TB incident cases in 2012 included about 1 million (13%) people who were co-infected with HIV. Altogether, 37% of the estimated TB/HIV co-infected cases resided in the WHO African Region countries, and collectively accounted for 75% of all TB/HIV co-infections worldwide. However, the estimated percentage of people living with HIV has remained steady over the recent years at 13% worldwide. About three-quarters of the deaths in 2012 occurred in the African and South-east Asian Regions, with India and South Africa accounting for nearly one-third of the global fatalities. About one-half of the TB patients in some African countries are additionally infected with HIV. With regard to gender, 34% of the estimated 8.6 million cases in 2012 were among women with the African and South-east Asian Regions accounting for 68% of the cases. Of the ~410,000 female deaths in 2012 nearly one-half occurred among HIV-positive cases (WHO, 2013).

The global notification of TB among children (≤15 years) was estimated to be 530,000 new cases, representing about 6% of the global incidence cases. An estimated 74,000 HIV-negative children with TB died in 2012, accounting for about 8% of the total estimated deaths. The case fatality rates among HIV-positive children were not available (WHO, 2013). The detrimental association between TB and HIV

appears to potentiate each condition in many aspects such as pathogenesis, epidemiologic profile, clinical presentation, treatment and prevention, not to mention the associated socio-economic issues. This is clearly evidenced by the fact that all areas with high TB cases are also high HIV-prevalent countries (Nunn *et al*., 2007). This is further substantiated by the observation that HIV infection is the major risk for progression of latent TB infection into active disease and that risk of developing active TB is significantly higher among TB/HIV co-infected patients (10% annual risk) compared to those solely infected with *M. tuberculosis* (8–10% lifetime risk) (Bloom and Murray, 1992). Although HIV-positive TB patients are generally shown to be less infectious than their HIV-negative counterparts (Cruciani *et al*., 2001), the mortality rate among this group is comparatively very high, i.e. 13.7% versus 0.5%, respectively (Murray *et al*., 1999).

Apart from the above, HIV infection alters the clinical picture of HIV-positive TB patients. For example, there is a high rate of false-positive skin tests among HIV patients (Barnes *et al*., 1991; Syed Ahamed Kabeer *et al*., 2009). In addition, atypical chest X-ray findings and/or TB patients with sputum smear negative results are not uncommon among HIV patients (Corbett *et al*., 2003; Mendelson, 2007; Hanekom *et al*., 2010). TB/HIV co-infected patients also frequently tend to suffer from extrapulmonary TB (Fätkenheuer *et al*., 1999) predominantly caused by opportunistic non-tuberculous mycobacteria such as the *M. avium* complex, *M. chelonae*, *M. fortuitum* and *M. kansasii* (Brennan and Nikaido, 1995). In a recent study, HIV infection has been shown to alter Cluster of Differentiation (CD)4 T cell memory phenotype among extrapulmonary TB patients (Matthews *et al*., 2012). The use of some TB drugs such as thiacetazone is contraindicated in HIV-infected patients due to some adverse side effects, thereby reducing the already limited options for proper management of TB (Kuaban *et al*., 1997) especially if one considers that the highest burden of TB is in settings that also have high prevalent rates of HIV. Because of these reasons, HIV has for some time been considered to be the most important single predictor of TB incidence in Africa (Corbett *et al*., 2003).

Treatment of TB

Tuberculosis is now a fairly curable disease for which effective treatment is available globally. The aims of TB treatment are to cure the patient and restore quality of life and productivity, prevent death from active TB or its late effects, prevent relapse, and prevent development and transmission of drug resistance (WHO, 2010).

The drugs are grouped mainly in two categories, namely first-line and second-line drugs, depending on their application. First-line drugs are administered for 6 or 8 months in what is commonly referred to as 'short-course chemotherapy' (SCC). This strategy targets treatment of drug-susceptible TB and is divided into two phases: the intensive phase and the continuation phase. The intensive phase covers the first 2 months of treatment and aims to kill actively growing and semi-dormant bacilli, thereby reducing the duration of infectiousness of an individual. The continuation phase lasts between 4 and 6 months depending on disease site and drug combination used and is intended to eliminate bacilli that are still multiplying and also reduce the risk of failure and relapses. In general, four categories of treatment can be distinguished according to the diagnostic status of the patient (smear positivity and treatment history). Within each category various options exist. The type of regimen used in a particular country depends on affordability, coverage by public health services and competence of the staff at a peripheral level (WHO, 2010).

First-line Drugs

The first-line drugs are mainly bactericidal and combine a high degree of efficacy with a relative toxicity to the patient during treatment and are mainly used in the treatment regimens of non-MDR-TB. They comprise isoniazid (H), rifampicin (R), streptomycin (S), ethambutol (E) and pyrazinamide (Z) (WHO, 2010).

H acts by inhibiting mycolic acid synthesis (Winder and Collins, 1970). Mutations in the *kat*G gene that encodes the catalase-peroxidase that activates the prodrug have been the most

frequently associated with H resistance (75–85%) (Garcia de Viedma, 2003). R, on the other hand, inhibits RNA synthesis by binding to the β-subunit of the RNA polymerase (Musser, 1995), and is the most potent sterilizing agent of all the first-line drugs. Mutations in the *rpo*B gene account for >98% of R-resistant isolates (Traore *et al*., 2000). Resistance to R is also commonly used as an indicator for MDR-TB (Cho *et al*., 2013). Together, H and R constitute the most powerful bactericidal TB drugs and are active against all populations of the TB bacilli.

S is bactericidal against rapidly multiplying TB bacilli. It acts by inhibiting protein synthesis and damaging cell membranes, which results in the death of the bacteria. Mutations in the *rrs* and *rps*L genes account for 65–75% resistance to S (Finken *et al*., 1993).

E is bacteriostatic and has a synergistic action with more powerful drugs to prevent the emergence of resistant bacilli. It inhibits the synthesis of the cell wall by interfering with the transfer of D-arabinose into cell wall arabinogalactans (Mikusova *et al*., 1995). Arabinogalactans are complex branched polysaccharides that connect mycolic acids to the inner peptidoglycan of the cell wall (Brennan and Nikaido, 1995). Mutations in the *emb*CAB operon coding for different arabinosyl transferases account for about 70% of resistant strains (Garcia de Viedma, 2003).

Z is bactericidal but is only active in an acid intracellular environment. It acts by inhibiting mycolic acid synthesis (Zimhony *et al*., 2000). Resistance to Z is mediated via mutations in the *pnc*A gene, encoding for pyrazinamidase (Scorpio and Zhang, 1996).

Fixed-dose TB Tablets

The WHO has developed and recommended formulations of a model list of essential anti-TB drugs and fixed-dose combinations (FDCs) of drugs (www.who.int/medicines/publications/essentialmedicines/en). FDC tablets contain different combinations of drugs, such as HR, HE, HRZ and HERZ. The efficacy of these FDCs has been shown to be comparable to the single tablet regimens, at least in smear-positive pulmonary TB patients (Bartacek *et al*., 2009). Some of the advantages of using FDC tablets include preventing the development of drug resistance, simplification of treatment and management, and reduction of misuse of the drugs for treatment of conditions other than TB. The main disadvantage with FDC tablets lies in the difficulty in handling side effects.

The WHO has also issued recommendations for treating TB in persons living with HIV. The recommendations state that TB patients with known positive-HIV status and all TB patients living in HIV-prevalent settings should receive daily TB treatment at least during the intensive phase and if possible for the continuation phase (Hopewell *et al*., 2006; WHO, 2010).

Second-line Drugs

Second-line drugs are reserve drugs that are only used in situations where the first-line drugs are failing. In general, they are less effective than first-line drugs but are more toxic, more expensive and require lengthy periods of administration of up to 2 years. They include fluoroquinolones such as ciprofloxacin and ofloxacin, which act by inhibiting type II topoisomerase (Wang, 1996) and aminoglycosides like kanamycin and amikacin (Edson and Terrell, 1999). Other drugs included in this category are viomycin, capreomycin (Herr and Redstone, 1966), ethionamide and para-aminosalicyclic acid. Other drugs include gatifloxacin, moxifloxacin and dyarilquinoline (Andries *et al*., 2005). Streptomycin is the oldest and perhaps the only drug that is used to treat drug-susceptible TB and extensively drug-resistant TB.

Acquisition of Drug Resistance

The increasing level of resistance to available TB drugs has necessitated the repurposing of old drugs and development of new anti-TB drugs. Drug resistance is a result of acquisition of spontaneous genetic mutations that occur naturally in individual mycobacteria.

Typically, the rates at which these mutations are acquired are so low that this mechanism would not lead to the clinical drug resistance of *M. tuberculosis* to TB drugs that is seen today. For example, the natural rate at which H and R acquire mutations is 3.5×10^{-6} and 3.1×10^{-8}, respectively. It is thus perceived that much of the resistance we normally encounter may be attributed to irregular drug intake due to either non-compliance or availability, poor quality of drugs in some instances and co-infection with non-tuberculous mycobacteria (NTM). The prolonged exposure to a single drug or suboptimal therapy may lead to the selection and expansion of resistant Mtb strains. In addition, the possibility of acquiring double spontaneous mutations to H and R, in the above example, is very low (9×10^{-14}). This suggests that resistance to more than one drug will most likely occur following sequential acquisition of mutations of different drugs as a result of sustained treatment failure (Traore *et al*., 2000).

Hope of New TB Agents

There has been an enhanced effort by many drug companies to invest in TB drug development. Presently, at least ten new or repurposed TB drugs are in the late phases of clinical development. In 2012, bedaquiline became the first new drug in a long time to be approved by the Federal Drug Administration (FDA) in the USA for treating MDR-TB patients. A number of other drugs including linezolid, sutezolid (PNU-100480), PA-824, SQ-109 and AZD-5847 are in Phase II clinical trials (WHO, 2013). In addition, other drugs are already in Phase III trials. For example, studies that evaluated the substitution of H by moxifloxacin in the intensive treatment phase and used rifampetine in the continuation phase reported favourable results (Jindani *et al*., 2014). Two other trials investigating the use of gatifloxacin instead of E or the substitution of moxifloxacin by either E or I are in progress. A third trial is currently evaluating the use of delamanid (OPC-67683) for treatment of MDR-TB (WHO, 2013).

Worldwide Prevalence of Drug Resistance

The bulk of the available data on global TB drug resistance has been systematically collected by the Global Project on Anti-Tuberculosis Drug Resistance Surveillance that was instituted by WHO in conjunction with the International Union against Tuberculosis and Lung Disease (IUATLD) from 1994 onwards. The data show that drug resistance among *M. tuberculosis* isolates is ubiquitous (WHO, 1997, 2000, 2004, 2008, 2010; Pablos-Méndez *et al*., 1998; Espinal *et al*., 2001; Aziz *et al*., 2006; Wright *et al*., 2009). Data exist from two-thirds of the WHO's 193 member states with at least two data sets being contributed by 71 countries. According to the 2008 global data on drug resistance among new cases, drug resistance to at least one anti-TB drug ranged from 0% to 56.3% Baku city (Azerbaijan), while the corresponding MDR rates ranged from 0% to 22.3% in Baku city. On the other hand, resistance to at least one anti-TB drug among previously treated TB patients ranged from 0% to 85.9% in Tashkent (Uzbekistan) with the highest MDR rates being recorded in Baku city, Azerbaijan (55.8%) and Tashkent (60%). According to global estimates, any drug resistance ranged from 0% in three European countries to 85.9% in Tashkent, Uzbekistan. Data on extensively drug-resistant TB (XDR-TB) available from 11 countries showed that, by and large, the XDR proportions among MDR-TB were lower in Central and Western Europe, the Americas and in the Asian countries (range 0–30%). The data from nine countries of the former Soviet Union indicated approximately 10% of MDR-TB cases were also XDR ranging from 4% in Armenia to 24% in Estonia (WHO, 2008). The proportion of XDR cases in MDR-TB cases in the latter countries was more worrisome because the percentages are based on high absolute numbers of cases compared to the former countries where the absolute number of MDR cases were few.

More recent data show new MDR-TB rates that ranged from 0% to 28.9%, with the highest rate being reported in Murmansk (Russian Federation), while the percentage among previously treated cases varied from 0% to 65.1%. Of the 38 countries and territories

that reported XDR cases, more than ten cases of XDR-TB were reported in only 6 (15%) instances (Zignol *et al.*, 2012). Overall data showed MDR rates of 3.4% (95% CI:1.9–5.0) and 19.8% (95% CI:14.4–25.1) among new and previously treated patients, respectively (WHO, 2013).

In general, the trends show that most low-burden TB countries exhibit stable drug resistance rates and absolute numbers of TB cases but there are increasing MDR rates in the Baltic States and in countries of the Russian Federation (WHO, 2008).

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