INTRODUCTION

1.1. Burden of tuberculosis

Tuberculosis (TB) is the leading cause of death from a single infectious pathogen and overall ninth leading cause of death worldwide (WHO 2017 and http://www.who.int/mediacentre/factsheets/fs310/en/ accessed on 4th February 2018). Tuberculosis is reported to cause 1.3 million deaths among HIV-negative people and an additional 0.374 million deaths among people living with HIV worldwide (WHO 2017). The World Health Organization (WHO) also reported that an estimated 10.4 million people to be affected by TB in 2016 globally (WHO 2017). Emergence of drug-resistant tuberculosis (DR-TB) makes the situation difficult as drugs used to treat DR-TB are more toxic, more expensive; require a longer duration of treatment. The success rate of the treatment of extensively drug-resistant tuberculosis (XDR-TB) and multidrug-resistant tuberculosis (MDR-TB) is reported to be only 28% and 52% respectively, versus 83% in drug-susceptible new TB cases (WHO 2016a). Delay in diagnosis of the drug resistance pattern is a critical factor which subsequently leads to excessive delay in initiating appropriate treatment; effecting the transmission of DR-TB in the community further complicating the control. (Swaminathan et al., 2016). Therefore, early diagnosis and treatment of the DR-TB is essential for effective control of tuberculosis globally.

Tuberculosis was declared as a public health emergency by WHO in the year 1993 and called on all governments, in all parts of the world, to make TB control an immediate priority (http://www.who.int/tb/features_archive/mr_statement/en/, accessed on 4th February 2018). Since the WHO declaration, awareness about the disease increased that helped to prevent millions of cases of TB and deaths due to TB (WHO
The mortality rate due to TB has fallen by 45% since 1990 and TB incidence rates are reported to be decreasing in most parts of the world. WHO also reported that between 2000 and 2013, an estimated 37 million lives were saved through effective diagnosis and treatment for TB (WHO 2014a).

Though significant improvement have been achieved in the control of TB, given the quantum of problem TB poses globally and increase in the incidence of drug resistant tuberculosis, TB is considered as re-emerging public health problem (Sohail. 2006; Zaman. 2010) that has resulted in several countries declaring the TB as a national / regional emergency and calling for special attention. For instance, given the overburden of TB in African countries with consistent increase in the prevalence of TB and death of more than half a million people every year resulted in the WHO Regional Committee for Africa representing 46 Member States declaring the TB an emergency in African region in 2005 (http://www.who.int/mediacentre/news/releases/2005/africa_emergency/en/ accessed on 4th February 2018). Due to increasing burden of TB in India, recently, in 2014, India’s Union Health Ministry declared the TB as a National emergency (http://www.dnaindia.com/india/report-tuberculosis-a-national-emergency-harshvardhan-2016671, accessed on 4th February 2018) since there is an increase in the emergence of DR-TB in India. As per the latest report of WHO (2017) more than one fourth (27%) of the global TB patients live in India.
1.2. *Mycobacterium tuberculosis*

*Mycobacterium tuberculosis* is the causative pathogen of tuberculosis, which was discovered by Robert Koch on 1882 March 24 for which he was awarded Nobel Prize in 1905 (Keshavjee and Farmer, 2012). *Mycobacterium tuberculosis* is a slow growing intracellular bacterium capable of maintaining dormancy, with complex cell wall and genetic heterogeneity (Cole *et al*., 1998). Though *M. tuberculosis* is the major causative agent of tuberculosis, *M. africanum* is known to cause TB in humans in certain regions of Africa while *M. bovis*, *M. caprae* and *M. pinnipedii* cause TB in wild and domesticated mammals (Delogu *et al*., 2013). The doubling time of *M. tuberculosis* is 12–24 hours under optimal conditions (Delogu *et al*., 2013). *M. tuberculosis* cell envelope has a peculiar structure with lipids constituting about 40% of the cell dry mass. The unique structure and organization of the lipids render *M. tuberculosis* impermeability to biocides / noxious compounds and drugs; furthermore unique chemical composition and structure of the envelope play a fundamental role in the virulence of *M. tuberculosis* (Delogu *et al*., 2013; Jackson, 2014). Though *M. tuberculosis* is known to survive only in human system and has no known environmental reservoir (Comas *et al*., 2013; Pai *et al*., 2016), some researchers have reported the presence of *M. tuberculosis* in few animals including cattle and Indian elephants (Ocepek *et al* 2005; Alemayehu Regassa *et al*., 2008; Chen *et al*., 2009; Ameni *et al*., 2013; Mittal *et al*., 2014; Zachariah *et al*., 2017) indicating the potential challenge in controlling TB as the pathogen can sustain in other animal reservoirs. The present situation suggests that there is an urgent need for integrated ‘One Health’ approach that accounts for the interplay between humans, livestock, wildlife, and ecology in the epidemiology of zoonotic TB to develop methods and control/eliminate TB (Kaneene *et al*., 2014; Sternberg Lewerin, 2015).
1.3. Tuberculosis

General symptoms of active TB disease are fever, fatigue, lack of appetite and weight loss and in those patients with pulmonary TB disease, persistent cough and haemoptysis (coughing up blood) in advanced disease (Pai et al., 2016). Tuberculosis is classified into latent TB infection (LTBI) and active TB disease (Pai et al., 2016). The LTBI is asymptomatic and non-transmissible (Pai et al., 2016). About one third of the global population is reported to be latently infected with *M. tuberculosis* which corresponds to more than two billion individuals (Sohail, 2006; Barry et al., 2009). However, recently, Houben and Dodd (2016) re-estimated that approximately 1.7 billion individual is latently infected with *M. tuberculosis* globally using a mathematical modelling study in 2014 (Houben and Dodd.2016). Since it is known that the incidence of active TB cases is proportional to the number of persons latently infected (Trauer et al., 2014), they serve as the reservoir of the active TB population globally; therefore, it is important to address the LTBI burden appropriately for successfully controlling TB (Houben and Dodd.2016). The active TB disease is transmissible for which culture-based or molecular based diagnostics methods are available.

1.3.1. Risk factors and comorbidities of tuberculosis

Multiple factors including malnutrition, consumption of alcohol smoking, comorbidities including HIV infection and diabetes conditions increase the risk of developing active TB disease. (http://www.who.int/tb/areas-of-work/treatment/risk-factors/en/, last accessed on 4th February 2018). Additionally, young age, indoor air pollution, use of immunosuppressive drugs, socioeconomic and behavioral factors have also been reported to increase the susceptibility to infection (Narasimhan et al., 2013).
Particularly, comorbid conditions of TB with HIV or diabetes add another dimension in the complexity of tuberculosis. For example, people living with HIV are more likely to develop TB than people without HIV; further, about one third of deaths among HIV-positive people are reported to be due to TB (http://www.who.int/mediacentre/factsheets/fs104/en/, last accessed 4th February 2018). Furthermore, prevalence of diabetes mellitus (DM), among TB patients is reported to be very high; for example, 25.3% of TB patients from India (Viswanathan et al., 2012); 12.3% of TB patients from Nigeria (Ogbera et al., 2015) and 12% of TB patients from China (Mi et al., 2013) were reported to be diapetic. TB and DM are known to make a deadly combination as they aggravate the clinical course of DM and TB respectively among people who are affected by both TB and DM (http://apps.who.int/iris/bitstream/10665/44698/1/9789241502252_eng.pdf, last accessed on 4th February 2018 and http://www.who.int/tb/areas-of-work/treatment/risk-factors/en/, last accessed on 4th February 2018). The emergence of drug resistant forms of TB and other comorbid conditions clearly indicate that the urgent need for novel measures for the prevention and management of TB.

1.3.2. Diagnosis of tuberculosis

Active tuberculosis disease can be diagnosed using four different techniques namely, X-ray, sputum smear microscopy, culture based methods and molecular tests. However, when chest X-ray is used, microbiological methods are used additionally to support the diagnosis. Among these four methods, sputum smear microscopy has been the cornerstone of TB diagnosis (Kik et al., 2014), and it is the most widely used for the diagnosis of active TB disease especially in low-income and middle-income countries (Pai et al., 2016). Though microscopy based method is inexpensive and easy to perform
with a limited infrastructure, its sensitivity is low and it cannot be used for the diagnosis of the drug resistant TB (DR-TB) (Kik et al., 2014). Recently, one of the molecular method, Xpert MTB/RIF also known as GeneXpert (Cepheid Inc., Sunnyvale, California, USA) was conditionally approved by the WHO (WHO 2014b) for the diagnosis of TB and rifampin resistant TB. The GeneXpert is a fully integrated and automated system consists of an instrument, personal computer, barcode scanner and preloaded software; single-use disposable cartridges contain lyophilized reagents, buffers and washes; target detection and characterization is performed in real time using a six-colour laser-detection device; and the assay provides results directly from sputum in less than 2 hours; the Xpert MTB/RIF can detect *M. tuberculosis* as well as mutations that is known to cause rifampin resistance (WHO 2014b)

World Health Organization has also conditionally recommended the use of two rapid diagnostic tests, LPA (line probe assay) to detect resistance against first-line drug and SL-LPA (second line LPA) to detect resistance to fluoroquinolones and to the second-line injectable drugs for patients with confirmed rifampin-resistant TB or MDR-TB (WHO 2016b and WHO 2016c) However, these molecular methods are limited to few drugs and their accuracy is relatively low compared with phenotypic drug susceptibility assay for the diagnosis of drug resistant tuberculosis. Recently whole genome sequencing based methods have been reported to be promising for the rapid diagnosis of drug resistant tuberculosis (Swaminathan et al., 2016). The effective way to control or reduce the burden of DR-TB would largely depend on the early diagnosis and initiation of appropriate treatment regimen promptly.
1.3.3. An overview about anti-tuberculosis therapy

Most of the currently used drugs for the treatment of tuberculosis were discovered more than 50 years and the chronological development of the anti-tuberculosis therapy (ATT) is provided in the Figure 1.1. The chronological development of TB drugs can be summarized as following: Streptomycin (STR) was the first drug to be discovered in 1943 for the treatment of TB. Later in 1948, paraaminosalicylic acid (PAS) and later Isoniazid (INH) (1952) were introduced for the treatment of TB. Due to the emergence of drug resistance in *M. tuberculosis* in the early periods, combination therapy was proposed. In the year 1952, a successful regimen of STR, PAS and INH were administered for 24 months. In 1960s, PAS was replaced by Ethambutol (EMB) which led to a reduction in the treatment period from 24 months to 18 months. In 1963, Rifampin was discovered and included in the TB treatment regimen (STR, INH, EMB, RIF) in 1970s which cured 95% of TB patients in 9-12 months of time. Pyrazinamide (PZN) is another important drug discovered in the early 1980s which replaced the STR in the treatment regimen (INH, EMB, RIF, PZN); this regimen cured the TB with 6-8 months of treatment. From 1980s, the four drug regimen (INH, EMB, RIF, PZN) is administered for the first 2 months (intensive phase) and two drug regimen (INH and RIF) for 4 months (continuation phase) as recommended by WHO (Ma and Lienhardt. 2009). Furthermore, in populations with high levels of isoniazid resistance including in India. INH and RIF is given along with EMB in the continuation phase. (WHO 2010 and WHO 2014a).
Figure 1.1: History of drug discovery and regimen development, adopted from Ma and Lienhardt. 2009
1.3.4. Current drug discovery pipeline for tuberculosis

The present global drug pipeline contains several promising candidate drugs, evaluated at various phases of preclinical and clinical development (http://www.newtbdrugs.org/pipeline.php, last accessed on 4th February 2018). Among them, bedaquiline and delamanid were recently approved provisionally for treatment of DR-TB (Mdluli et al., 2015) and evaluated in phase 3 for the treatment of DR-TB. Additionally, various regimens are also being evaluated for the treatment of DR-TB; for example, a regimen containing pretomanid - moxifloxacin – pyrazinamide is being evaluated for the treatment of MDR-TB (http://www.newtbdrugs.org/pipeline/clinical, last accessed on 4th February 2018). Further, a novel regimen, Nix-TB containing bedaquiline, pretomanid and linezolid is evaluated in phase 3 to cure the XDR-TB (extensively drug-resistant TB); currently, a phase 3 clinical trial evaluating the Nix-TB is ongoing in South Africa (http://www.tballiance.org/portfolio/trial/5089, last accessed on 4th February 2018 https://clinicaltrials.gov/ct2/show/record/NCT02333799, last accessed on 4th February 2018) is in a phase 3 clinical trial. Since all the drugs included in the Nix-TB are injection-free and has potential to reduce the treatment period, Nix-TB is believed to improve the treatment of XDR-TB if the clinical trial turns out to be successful (Swaminathan et al., 2016). In the development pipeline, four drugs namely, SQ109, LCB01-0371, PBTZ-169 and nitazoxanide are being evaluated in the phase 2 clinical trial while six candidate drugs namely TBA-7371, Q203, GSK 070, GSK 3036656, OPC-167832 and TBI-166 are in the phase 1 clinical trials for the treatment of TB (https://www.newtbdrugs.org/pipeline/clinical, last accessed on 4th February 2018). Three more candidate drugs, PBTZ 169, BTZ 043 and Spectinamide 1810 are in the preclinical trials. High-throughput screening to identify novel inhibitors (Ananthan

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et al., 2009; Sipos et al., 2015) against *M. tuberculosis* is also encouraging and strengthening the discovery pipeline.

1.3.5. Vaccines for prevention of tuberculosis

The BCG (*Mycobacterium bovis* bacilli Calmette–Guérin) is the only vaccine currently used and available against TB (Andersen and Doherty, 2005). BCG was demonstrated to be protective against TB after a successful clinical trial conducted during 1921 to 1927 (Nunes-Alves et al 2014; Andersen and Doherty, 2005; Plotkin and Plotkin, 2011). BCG vaccine was developed by 230 serial passages of *Mycobacterium bovis* for a period of more than 13 years; thus, it is an attenuated (avirulent) strain of virulent *Mycobacterium bovis*. In TB endemic countries, newborns are immunized as soon as possible after birth with a single intradermal dose of BCG; it is well known that BCG protects against meningeal TB and from disseminated forms of TB in children (Montagnani et al., 2014) though its efficacy to protect adult varies from nil to 80% (Andersen and Doherty, 2005; Montagnani et al., 2014;). Since, BCG protects children, but do not vaccinated individuals throughout their life time, more efficient vaccine than the BCG is needed. At present, a total of 13 candidate vaccines are being evaluated in various stages of clinical research (Pai et al., 2016; Fletcher and Schrager, 2016). Research to develop novel vaccines capable of preventing the infection of *M. tuberculosis* or preventing active TB disease among the latently infected individuals (LTBI) so as to prevent the transmission of the *M. tuberculosis* will be a key to the control and subsequent elimination of the TB.
1.3.6. Challenges in successful control and elimination of tuberculosis

Despite several advancements in the last two decades from rapid diagnosis of TB and drug resistant TB, introduction of two new drugs for the treatment of DR-TB, and evaluation of multiple candidate vaccines in the clinical research pipeline there are many challenges ahead to be overcome in successfully controlling and eliminating the TB. Major challenges in the TB can be summarized as given below:

1) **Tuberculosis is the top most killing infectious diseases:** TB killed 1.5 million people including 1.3 million HIV-negative and 0.374 million HIV-positive TB patients in 2014 (WHO 2017). The death toll included 890,000 men, 480,000 women and 140,000 children.

2) **High burden of TB-incidence:** Further, 10.4 million people were reported to be infected by TB in 2016 globally and majority of the incident cases (56%) were reported to occur in a total of five countries, namely India, Indonesia, China, the Philippines and Pakistan (WHO 2017).

3) **Missing cases:** The fact that new cases went undiagnosed or were not reported indicates that the quality of care for these people is unknown (WHO 2015). In this line, unreported MDR-TB patients are also is a major concern.

4) **Lengthy duration of treatment:** The treatment for TB (drug sensitive TB: DS-TB) takes six months of time which comprise of INH, RIF, EMB and PYZ for two months (intensive phase) and INH, RIF and EMB (WHO 2014a) for following four months (continuation phase). However, in case the patient is reported to have DR-TB (either MDR-TB/XDR-TB), second-line drugs and other drugs are used for a period of 24 months for successful treatment.
5) Complexity in treatment of DR-TB:
   a. Lengthy treatment
   b. Relatively more toxic drugs
   c. Relatively less effective than first line drugs
   d. Poor success rate of treatment than in the DS-TB.

6) Latent TB: *M. tuberculosis* establishes latency in the host without any symptoms and physical signs, causes no obvious disturbance and is not recognized by the physician. Further there are no standard method yet available to differentiate among the latently infected individuals who will develop active TB in the future.

7) No protective vaccine available for tuberculosis: Though BCG vaccine is available, it partly protects children from TB. BCG does not provide protective immunity in adults. Lack of vaccine that can protect the host for lifelong is the major setback in the elimination of TB.

8) Co-infection with HIV: Co-infection of TB and HIV is also the major concern since people living with HIV are known to have more chances of developing TB and about one third of deaths among HIV-positive people are also known to be due to TB (http://www.who.int/mediacentre/factsheets/fs104/en/, last accessed 4th February 2018).

9) Co-morbidity with Diabetes: TB among diabetes makes a deadly combination as both of these clinical conditions known to worsen the clinical course of each other (http://apps.who.int/iris/bitstream/10665/44698/1/9789241502252_eng.pdf, last accessed on 4th February 2018).
10) **Reported animal reservoirs:** Incidence of *M. tuberculosis* in animals including cattle and elephants has been recently reported from several countries enabling the pathogen to have reservoirs (Ocepek *et al.* 2005; Alemayehu Regassa *et al.*, 2008; Chen *et al.*, 2009; Ameni *et al.*, 2013; Mittal *et al.*, 2014; Zachariah *et al.*, 2017); this is another potential threat in the control of tuberculosis.

1.3.7. Potential solutions for the successful control and elimination of tuberculosis

Broad understanding about the challenges in controlling TB has shed more light on what needs to be done that can enable us to control and subsequently eliminate TB successfully. These include:

1. Rapid diagnostic methods to determine the drug resistant profile on the same day of diagnosis of TB so as to decide the precise curative regimen for patients.
2. Methods to determine who will develop active TB disease from among those who have LTBI (latent TB infection).
3. Novel treatment regimen containing very few number of drugs which can efficiently cure TB with minimum doses and within minimum number of days compared to the currently used extended regimen six months for the treatment of drug sensitive TB and 24 months for the treatment for DR-TB. (https://www.cdc.gov/tb/topic/treatment/tbdisease.htm last accessed on 4<sup>th</sup> February 2018).
4. Efficient preventive vaccine which can protect the individuals for lifetime unlike the currently available BCG that protects children but fail to protect, in general, adults from TB.
1.3.8. *Mycobacterium tuberculosis* H37Rv

The H37Rv strain of *M. tuberculosis* has retained full virulence in animal models of tuberculosis, unlike some clinical isolates; it is also susceptible to drugs and amenable to genetic manipulation; thus it is being used for laboratory studies (Cole *et al.*, 1998). H37Rv strain of *M. tuberculosis* is being used extensively worldwide to understand various aspects of the biology of the pathogen including genomics, pathogenesis, drug-resistance and to identify targets for the discovery of novel drugs, diagnostics and vaccines. The first complete genome of *M. tuberculosis* H37Rv was sequenced in 1998 (Cole *et al.*, 1998). The researcher found *M. tuberculosis* genome to comprise 4,411,529 base pairs with a G + C content of 65.6% and contains about 4000 genes; in the first analysis, *M. tuberculosis* genome was reported to contain fifty genes coding for functional RNA molecules, 3924 genes encoding proteins; the circular map of the *M. tuberculosis* genome is illustrated in Figure 1.2 (Cole *et al.*, 1998). Later on, Cole and his research team re-annotated the complete genome of the *M. tuberculosis* and found 82 more genes to be present in the genome; however no change was reported in the number of functional RNA genes (Camus *et al.*, 2002).
Figure 1.2: Circular map of the chromosome of *M. tuberculosis* H37Rv adopted from Cole *et al.*, (1998). The color coding provided in the figure has been adopted from the original source article (Cole *et al.*, 1998): the outer circle shows the scale in Mb, with 0 representing the origin of replication. The first ring from the exterior denotes the positions of stable RNA genes (tRNAs are blue, others are pink) and the direct repeat region (pink cube); the second ring inwards shows the coding sequence by strand (clockwise, dark green; anticlockwise, light green); the third ring depicts repetitive DNA (insertion sequences, orange; 13E12 REP family, dark pink; prophage, blue); the fourth ring shows the positions of the PPE family members (green); the fifth ring shows the PE family members (purple, excluding PGRS); and the sixth ring shows the positions of the PGRS sequences (dark red). The histogram (centre) represents G + C content, with, <65% G + C in yellow, and >65% G + C in red.