# Tuberculosis in India: the continuing challenge

**Rajpal S. Kashyap[1,*], Amit R. Nayak[1], Aliabbas A. Husain[1], Hari M. Gaherwar[1], Hemant J. Purohit[2], Girdhar M. Taori[1] and Hatim F. Daginawala[1]**

[1]Biochemistry Research Laboratory, Central India Institute of Medical Sciences, Nagpur 440 010, India
[2]Environmental Genomics Unit, National Environmental Engineering Research Institute, Nehru Marg, Nagpur 440 020, India

**Despite many efforts from health policy makers (local and international societies) to eradicate tuberculosis (TB), India continues to account for majority of overall TB cases and higher mortality rates as compared to global scenario. The factors such as hygiene, overcrowding, under nutrition, lack of awareness, non-availability of rapid diagnostic tool and poor efficacy of TB vaccine, are the major reasons why India continues to be among countries accounting for highest TB burden cases annually. To bring this disease under control we need to work on these issues discussed briefly in the present article.**

**Keywords:** BCG vaccine, drug resistance, diagnosis, tuberculosis.

## Introduction

TUBERCULOSIS (TB) is possibly as ancient as the human race. In earlier days it was called 'consumption disease' as it would gradually consume human body[1]. TB is a contagious and airborne disease caused by a group of closely related bacterial species termed the *Mycobacterium tuberculosis* complex (MTBC)[2]. It is presumed that the genus *Mycobacterium* originated more than 150 million years ago[3]. MTB is regarded as a human specialized form of *M. bovis* which is believed to be transmitted by milk drinking Indo-Europeans during their migration into Western Europe and Eurasia[4].

## Global epidemiology

TB remains a major global health problem with almost one third of global population believed to be infected. According to the recent World Health Organization (WHO) report, there were 9 million new cases of TB reported in 2011, with 1.4 million deaths presenting a major impediment to TB control[5]. Around 12% of worldwide TB cases are HIV associated. TB is also major hindrance in socioeconomic development with 75% of people infected being in economically productive age group of 15–54 years. 95% of all cases and 99% of deaths occur in developing countries, with the greatest burden in sub-Saharan Africa and South East Asia[6].

## Indian overview

TB in India is thought to have originated as early as 3300 years ago[3]. According to statistics, India is 17th among 22 high burden countries in terms of TB incident rates[7]. Figure 1 depicts estimated incidence, prevalence and death rate due to TB in India. Although the pulmonary system is the most common location for TB, extra pulmonary TB (EPTB) disease, resulting from spread of MTB from lungs to other tissues and organs through bloodstream, occurs in more than 20% of immunocompromised patients. In India and other developing countries, lymph node TB is among a major form of EPTB caused by non-tuberculous mycobacteria (NTM) species[8]. Central nervous system TB such as tuberculous meningitis (TBM) is among the most fatal forms accounting for 70–80% of all neurological TB cases[9]. Lack of awareness to a variety of forms of EPTB, along with its asymptomatic conditions has made EPTB difficult to be diagnosed and is the reason that it has remained among a major infectious killer in Indian population.

High prevalence of latent TB infection (LTBI) in developing countries like India has further complicated the diagnostic scenario and is also regarded as a major threat due to its reversion into active TB. An estimated 40% of the Indian population is infected with TB bacteria, a vast majority of whom are latently infected rather than by active TB[10]. Our study was carried out in one particular community in high TB-endemic regions in Nagpur district, where we observed that a majority of population screened were latently infected, with high exposure to active TB cases. In another study carried out in malnourished tribal population of Melghat region, we noted that from among 600 participants, only 9% cases were diagnosed with TB. Further studies based on results of interferon gamma release assays (IGRAs) and tuberculin skin test (TST), it was revealed that more than half of the population was latently infected with MTB. These observations highlighted the need for a major initiative to be taken in remote and under developed high TB endemic settings to diagnose LTBI cases. Subsequent follow-up of latent cases to monitor sero conversion along with timely and

*For correspondence. (e-mail: raj_ciims@rediffmail.com)

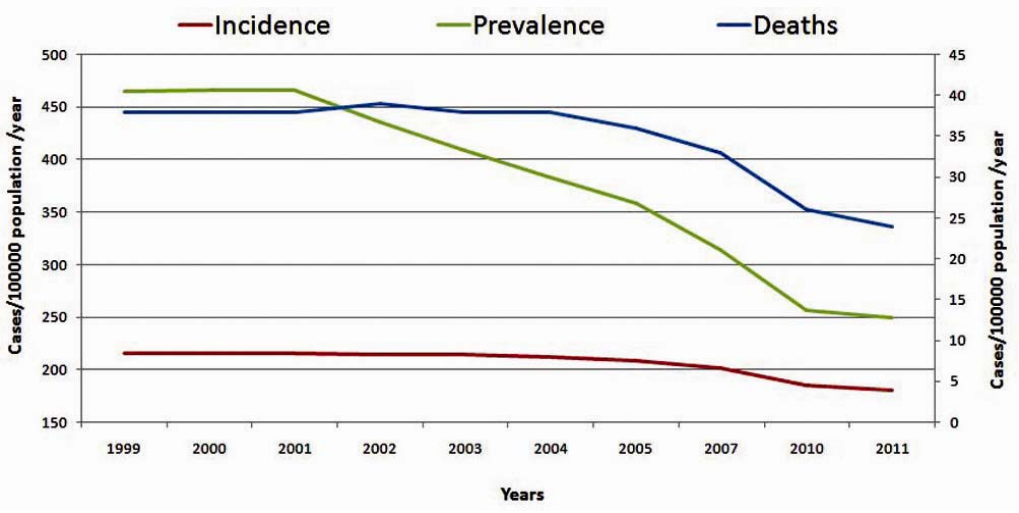


**Figure 1.** Tuberculosis (TB) cases in India from 1999 to 2011.

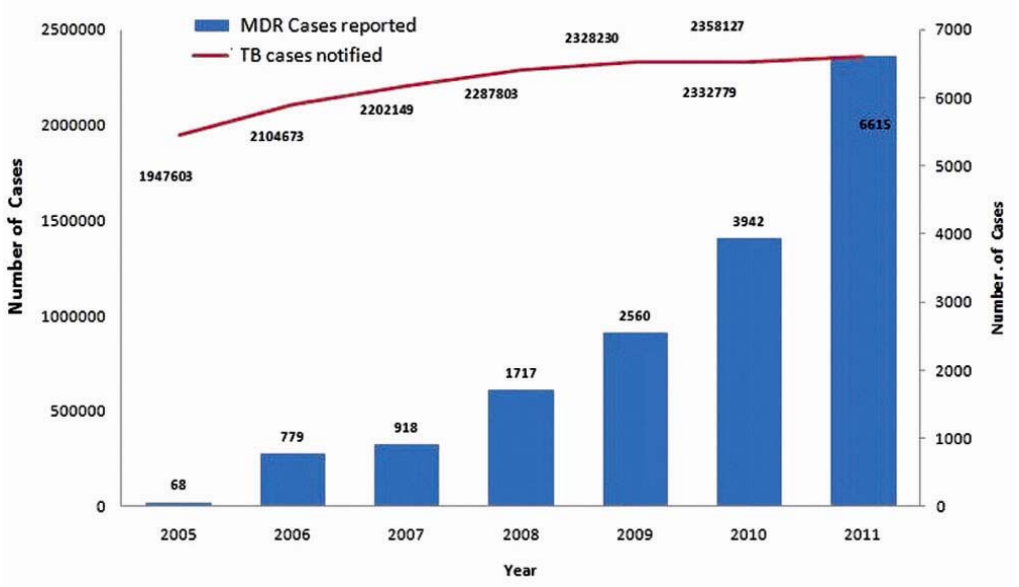


**Figure 2.** Annual multi-drug resistance (MDR) cases reported in India along with prevalence rate of TB notified. Numbers above the bars indicate total number of cases of MDR-TB reported each year. Numbers above the red line notifies the estimated total TB cases reported each year[5].

proper treatment in diagnosed cases may help in minimizing burden of TB.

Another major reason for high TB prevalence in Indian population is emerging drug resistance. According to WHO report, India is home to 73,000 patients with multiple-drug resistance (MDR) TB[5]. Recent statistics showed that although prevalence of TB has remained the same, cases of MDR are on a rise with each passing year (Figure 2). Limitations in TB drugs management and variation or mutation at MTB strain level have given rise to a more deadly and contagious form of drug resistance strain known as extensive-drug resistant tuberculosis (XDR-TB). Recently, a case of total drug resistance (TDR) was also reported, however it was not confirmed[11]. On the other side, efficacy of anti-TB treatment (ATT) drugs in case of EPTB has further aggravated the issue. For instance in TBM, drug given to patients has to cross blood brain barrier, and it is not clear what concentration of drugs reaches localized area which complicates the treatment.

Various programmes have been implemented by policy makers, government authorities, local and International health organizations to eradicate TB. Implementation of the Revised National Tuberculosis Control Programme (RNTCP) involving Directly Observed Therapy Short (DOTS) course in 1993 is regarded as the most successful

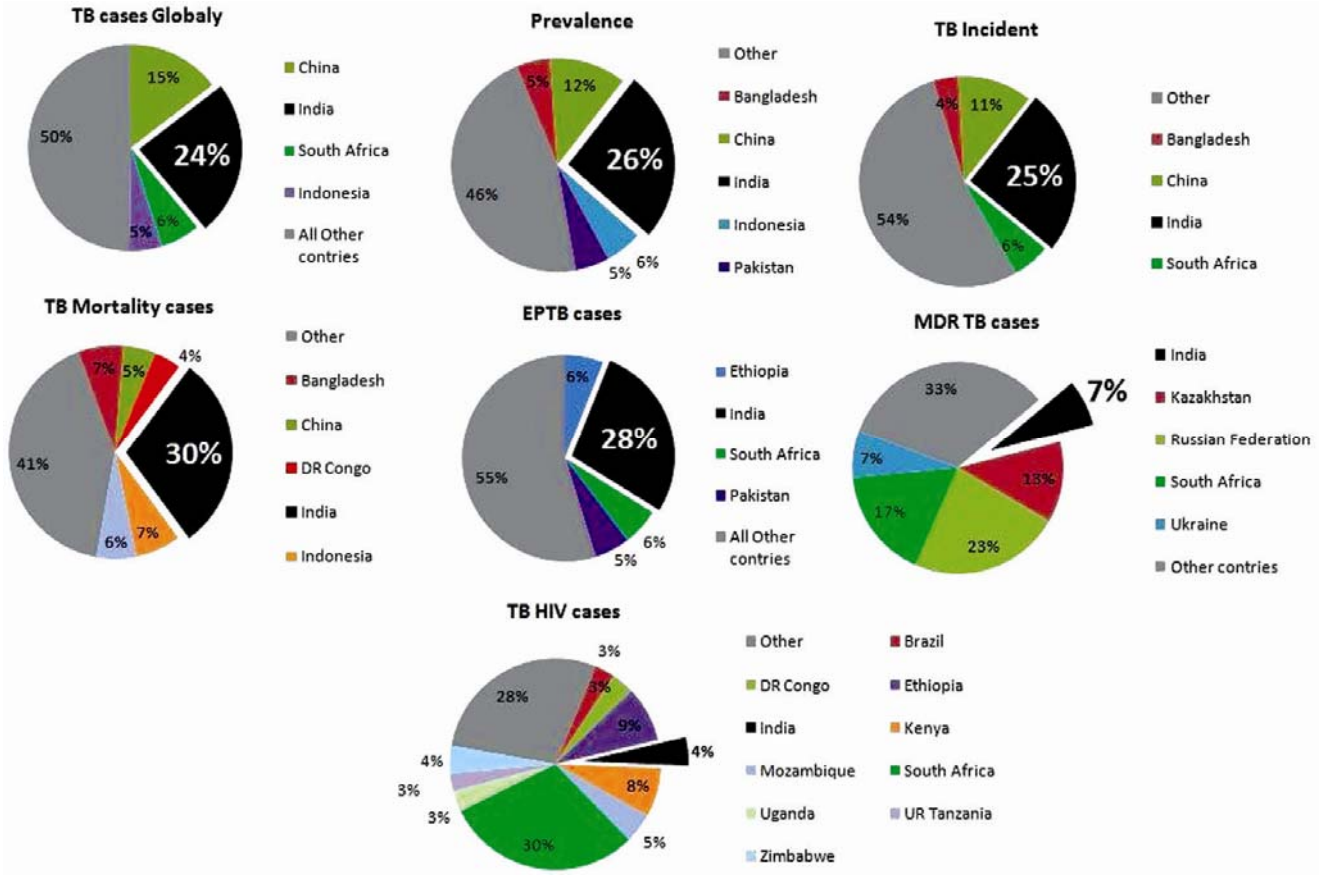


**Figure 3.** Recent estimates of TB in India (region in black) in comparison with global TB cases[5].

TB control programme in India, with 100% nationwide coverage rates achieved by March 2006 (ref. 7). Recent report of RNTCP suggests that total cases of TB have come down to almost half in the country since its implementation[7]. Despite all these efforts, according to current WHO estimates, India continues to account for majority of TB cases, along with high incidence and prevalence rate, more cases being of EPTB and higher mortality rates as compared to global scenario (Figure 3). TB is regarded as a disease of poverty which is impeccably associated with overcrowding and under nutrition. All these factors are the major reasons why India continues to be among countries accounting for highest burden of TB cases annually[7].

## TB diagnosis

Diagnosis of pulmonary TB (PTB) patients relied on simple chest-X ray showing regions of infiltration along with cavitations in lobes of the lungs. However, such finding is not observed in many cases, which may be one of the main drawbacks of this technique[12,13]. Demonstration of acid fast bacilli (AFB) in sputum of PTB patients with abnormal chest radiograph is another alternative available for diagnosis. However, sputum positivity observed in PTB cases is around 20–30%, thus questioning on the remaining 80% of the cases where AFB remains negative[14,15]. Demonstration of MTB in clinical specimen using mycobacteria-specific culture remains a gold standard technique in TB diagnosis[16]. However, culture of MTB can take up 6–8 weeks, and in 10–20% of cases the bacillus is not successfully cultured.

Nucleic Acid Amplification Test (NAAT) is regarded as the most promising tool in TB diagnosis. This technique is based on detecting both DNA and RNA, specific to MTB, using polymerase chain reaction (PCR) and is regarded as highly specific, overcoming most of the limitations of conventional culturing system. Despite recent advancement, sensitivity of PCR assays has been variable in diagnosis of TB. The sensitivity and specificity of various molecular assays used in diagnosis of TB are given in Table 1. There are various factors which might be contributing to this variability. The most important factor is copy number of mycobacterial gene targeted; high copy number of the target gene increases the chance of positive PCR reaction[17]. Another major reason for the variability is the variation in the amount of DNA extracted by various protocols used in different laboratories. A blind comparison study among seven laboratories found variable sensitivities (2–90%) using different protocols in

**Table 1.** Sensitivity and specificity of molecular tests used for the diagnosis of TB infection

| Type of TB | Gene target | Sensitivity (%) | Specificity (%) | References |
|---|---|---|---|---|
| PTB (Sputum) | IS6110 | 89.6 | 100 | Aryan *et al.*, 2013 |
| EPTB (Pleural fluid) | IS1081 | 48.6 | 83.3 | Yang *et al.*, 2011 |
| PTB (Sputum) | IS6110 | 87.9 | 98 | Lira *et al.*, 2012 |
| PTB (Bronchial wash) | IS6110 | 43.2 | 93.3 | Kheawon *et al.*, 2012 |
| PTB (Sputum) | IS6110 | 92.4 | 98 | Zakham *et al.*, 2012 |
| PTB (Sputum) | hsp 65 (165 bp) | 73.33–84.61 | 80 | A. Singh & V. Kashyap 2012 |
| | dnaJ (365 bp) | 73.33–84.62 | 80 | A. Singh & V. Kashyap 2013 |
| | IS6110 (541 bp) | 73.33–84.63 | 80 | A. Singh & V. Kashyap 2014 |
| PTB | IS6110 | 99 | 71 | Chakravorty *et al.*, 2005 |
| EPTB (Pleural fluid) | devR (Rv3133c) | 75 | 94 | Chakravorty *et al.*, 2005 |
| | IS6110 | 75 | 94 | Chakravorty *et al.*, 2005 |
| EPTB (Pleural tissue) | devR | 62 | 100 | Chakravorty *et al.*, 2005 |
| | IS6110 | 75 | 100 | Chakravorty *et al.*, 2005 |
| EPTB (Lymph node) | devR | 47 | 67 | Chakravorty *et al.*, 2005 |
| | IS6110 | 60 | 75 | Chakravorty *et al.*, 2005 |
| EPTB (Pleuritis) | hupB | 71 | 100 | Kumar *et al.*, 2010 |
| PTB | 23S rRNA | 64 | 100 | Varma-Basil *et al.*, 1994 |
| EPTB (Pleural fluid) | GC reach repetitive sequence | 63 | 93 | Verma-Basil *et al.*, 1995 |
| EPTB (TBM) | qRT (devR) | 88 | 92 | Haldar *et al.*, 2009 |
| | devR | 88 | 87 | Haldar *et al.*, 2007 |
| | IS6110 | 85 | 84 | Haldar *et al.*, 2009 |
| EPTB (TBM) | Rv0934 (38 kDa gene) | 90 | 100 | Kulkarni *et al.*, 2005 |
| EPTB (TBM) | IS6110 | 67 | 100 | Desai *et al.*, 2006 |
| EPTB (Abdominal TB) | Rv0934 (38 kDa gene) | 77 | 69 | Kulkarni *et al.*, 2006 |
| EPTB (Osteomyelitis TB) | IS6110 | 72 | 80 | Jambhekar *et al.*, 2006 |
| PTB | Rv0355 | 83 | 100 | Prasad *et al.*, 2001 |
| EPTB (Pleural fluid) | Rv0355 | 78 | 100 | Srivastava *et al.*, 2006 |
| EPTB (TBM) | Rv0355 | 70 | 100 | Srivastava *et al.*, 2001 |
| *M. tuberculosis* isolates | rpoB | 100 | 100 | Varma-Basil *et al.*, 2004 |
| EPTB (TBM) | IS6110 | 91 | 76 | Deshpande *et al.*, 2007 |
| EPTB (Pleural TB) | IS6111 | 69 | 66 | Nagdev *et al.*, 2008 |
| EPTB (TBM) | hupB | 61 | 88 | Shah *et al.*, 2006 |
| EPTB (Pleural TB) | mce3 operon | 69 | 100 | Kumar *et al.*, 2009 |
| PTB | mce3 operon | 96 | 100 | Kumar *et al.*, 2009 |
| PTB | hsp65 | 84 | 100 | Varma-Basil *et al.*, 2010 |
| PTB (Sputum) | IS6110 | 94.74 | 100 | Hasan *et al.*, 2012 |
| EPTB (tubercular meningitis, ascites, lymphadenitis) | hupB | 90.34 | 94.48 | Jain 2011 |
| EPTB (TBM) | *IS6110* | 84 | 92 | Nagdev *et al.*, 2010 |

spiked samples of PTB cases, therefore, further evaluation of such tests is needed[18]. Similarly sensitivity and specificity are also affected by the type of TB (i.e. PTB or EPTB) and the specimen sample type and volume used for DNA extraction[19].

In our laboratory we have evaluated the conventional PCR based on *IS6110* region (copy number 16) of MTB in both PTB and EPTB cases. We found that 91.4% of the culture-positive and 24.1% of the culture-negative, TBM cases were found to be positive by the IS6110 PCR assay[20]. Similarly, the peripheral blood-based PCR detection has also been found to be a reliable approach in the diagnosis of PTB in immunocompetent and immunocompromised individuals[21]. Although the test has high specificity (89–95%), sensitivity (20–90%) still remains variable in the diagnosis of PTB and EPTB. Apart from the sensitivity and specificity issues, another major aspect to be considered while using the molecular tools is that such techniques are unable to differentiate between live and dead bacteria (active or passive infection). Therefore further evaluation of molecular test with combinatorial approach, of analysing the molecular along with biomarker testing may offer the best diagnostic protocol for detecting active TB infection, with increased sensitivity and specificity.

Serological test was widely used for the diagnosis of TB. However, last few years have seen lots of controversy on serological tests, especially antibody detection. However studies have shown serodiagnosis as an important tool for MTB antigen and antibody detection and may be helpful in early diagnosis where other tests remain negative[22,23]. Cost effectiveness of serological test is a major advantage over molecular assays; however sensitivity and specificity may vary with stage of infection and type of test employed[24]. Antigen detection tests are particularly important and reliable in early stages of

**Table 2.** Sensitivity and specificity of serodiagnostic tests used for the diagnosis of TB infection

| Type of TB | Techniques | Protein antigens | Sensitivity (%) | Specificity (%) | References |
|---|---|---|---|---|---|
| EPTB (serum) | ELISA | Cocktail IC-Antigen (ES-31/ES/43/EST-6) | 97 | 100 | Harinath *et al.*, 2006 |
| EPTB (serum) | ELISA | Ab/Ag/IC-Ag | 96 | 88 | Upadhye *et al.*, 2007 |
| EPTB (serum) | ELISA | E57to R84 of Rv3872 | 90 | 100 | Mukherjee *et al.*, 2007 |
| EPTB (serum) | ELISA | Rv3878 antigen | 84 | 91 | Mukherjee *et al.*, 2007 |
| EPTB (urine) | ELISA | Lipoarabinomannan (LAM) | 33 | 100 | Lawn *et al.*, 2009 |
| EPTB (urine) | ELISA | Lipoarabinomannan (LAM) | 44 | 89 | Mutetwa *et al.*, 2009 |
| EPTB (urine) | ELISA | Lipoarabinomannan (LAM) | 51 | 88 | Reither *et al.*, 2009 |
| EPTB (urine) | ELISA | Lipoarabinomannan (LAM) | 20 | 83 | Daley *et al.*, 2009 |
| EPTB (urine) | ELISA | Lipoarabinomannan (LAM) | 59 | 96 | Shah *et al.*, 2009 |
| EPTB (urine) | ELISA | Lipoarabinomannan (LAM) | 13 | 99 | Dheda *et al.*, 2010 |
| EPTB (pleural) | ELISA | 16 kDa (HSP-X) alpha-crystalline | 63 | 95 | Kaushik *et al.*, 2012 |
| EPTB (pleural) | ELISA | Ag 85 complex antigen | 72 | 94 | Kashyap *et al.*, 2010 |
| EPTB (pleural) | ELISA | 65 kDa antigen | 88 | 95 | Kashyap *et al.*, 2010 |
| EPTB (pleural) | ELISA | 71 kDa antigen | 83 | 76 | Kashyap *et al.*, 2010 |
| EPTB (pleural) | ELISA | 14 kDa antigen | 100 | 76 | Kashyap *et al.*, 2010 |
| EPTB (TBM) | ELISA | Ag 85 complex antigen | 90 | 98 | Kahsyap *et al.*, 2005 |
| EPTB (TBM) | Cell ELISA | 30 kDa antigen | 92 | 92 | Kahsyap *et al.*, 2004 |
| EPTB (TBM) | SRID | Whole cell lysate (H37Rv) | 94 | 98 | Kahsyap *et al.*, 2002 |
| EPTB (TBM) | Dot blot | Whole cell lysate (H37Rv) | 85 | 95 | Kahsyap *et al.*, 2003 |
| EPTB (TBM) | ELISA | ESAT-6 antigen | 80 | 94 | Kahsyap *et al.*, 2009 |
| EPTB (TBM) | ELISA | 30 kDa antigen | 80 | 90 | Kahsyap *et al.*, 2004 |
| PTB (serum) | ELISA | ES-31+ES-41+ES-43 | 96 | 100 | Gupta *et al.*, 2005 |
| PTB (serum) | ELISA | Rv3872 antigen | 92 | 95 | Mukherjee *et al.*, 2007 |
| PTB (serum) | ELISA | Antigen cocktail (ES-31/ES-43/EST-6) | 91 | 97 | Harinath *et al.*, 2006 |
| PTB (serum) | ELISA | ES-31ES-43EST-6 | 77 | 90 | Majumdar *et al.*, 2010 |
| PTB (serum) | ELISA | IC-Ag ES-31/ES-43/EST-6 | 77 | 90 | Majumdar *et al.*, 2011 |
| PTB (serum) | ELISA | Antigenic epitope (E57to R84 of Rv3872) | 94 | 100 | Mukherjee *et al.*, 2007 |
| PTB (serum) | ELISA | Rv3872 antigen | 92 | 95 | Mukherjee *et al.*, 2007 |
| PTB (serum) | ELISA | Rv3878 antigen | 88 | 91 | Mukherjee *et al.*, 2007 |
| PTB (serum) | ELISA | 16kDa (HSP-X) alpha-crystaline | 80 | 95 | Kaushik *et al.*, 2012 |
| PTB (serum) | ELISA | Purified protein derivative (H37Rv) | 89 | 87 | Perkin *et al.*, 2003; Kailash *et al*., 1983 |
| PTB (serum) | ELISA | Antigen 5 (38 kDa) | 89 | 94–100 | Andersen *et al.*, 1989; Ma, Y. *et al.*, 1986 |
| PTB (serum) | ELISA | Cord factor | 81–84 | 96–100 | He, H. *et al.*, 1991; Maekura, 1993 |
| PTB (serum) | ELISA | ESAT-6 antigen | 67 | 51 | Greenaway *et al.*, 2005; Zhang *et al.*, 2007 |
| PTB (serum) | ELISA | CFP-10 antigen | 48–63 | 51–71 | Dillon *et al.*, 2000; Murthy *et al*., 2007 |
| PTB (serum) | ELISA | Kp90 antigen | 78 | 82 | Arikan *et al.*, 1998 |
| PTB (serum) | ELISA | 60 kDa antigen | 68–91 | 100 | Zou *et al.*, 1994; Gupta *et al.*, 1995 |
| PTB (serum) | ELISA | 30 kDa antigen | 84 | 96 | McDonough *et al*., 1992; Umadevi *et al*., 2003 |
| PTB (serum) | ELISA | MPT51 | 80 | 86 | Achkar *et al.*, 2006 |
| PTB (serum) | Flow cytometery | ESAT6 | 100 | 80 | Hughes, A. J. *et al.*, 2005 |
| PTB (serum) | ELISA, WB | ES-31/ES-41/ES-43 | 96 | 100 | Sonika Gupta *et al.*, 2005 |
| PTB (serum) | ELISA | Antigen 5 and PPD | 81 | 54 | Balestrino, E. A. *et al.*, 1994 |
| PTB (serum) | ELISA | PPD | 82 | 54 | Balestrino, E. A. *et al.*, 1994 |
| PTB (serum) | ELISA, WB | ESAT6, HSP-x, CFP-10, Ag85, PST-1, 30–38 kDa | 77 | 78 | Rum Shin, A. *et al.*, 2008 |
| PTB (serum) | QFT-IT, TSPOT-TB | IP10 and QFT-IT | 87 | 97 | Marten Ruhwald *et al.*, 2011 |
| PTB (serum) | ELISA | ESAT6 | 80–87 | 85 | Debasis Biswas *et al.*, 2002 |
| PTB (serum) | ELISA | 45 kDa/47 kDa | 96 | 73 | Chanteau, S. *et al.*, 1999 |
| PTB (serum) | ELISA | 38 kDa | 61 | 100 | Alamelu Raja *et al.*, 2007 |
| PTB (serum) | ELISA | 30 kDa | 62 | 100 | Alamelu Raja *et al.*, 2007 |
| PTB (serum) | ELISA | 16 kDa | 67 | 99 | Alamelu Raja *et al.*, 2007 |
| PTB (serum) | ELISA | 27 kDa | 57 | 98 | Alamelu Raja *et al.*, 2007 |
| PTB (serum) | ELISA | 38 kDa | 68 | 96 | Alamelu Raja *et al.*, 2007 |
| PTB (serum) | ELISA | 30 kDa | 68 | 97 | Alamelu Raja *et al.*, 2007 |
| PTB (serum) | ELISA | 27 kDa | 73 | 97 | Alamelu Raja *et al.*, 2007 |
| PTB (serum) | EIA | 30 kDa | 82 | 93 | Henritte Stavri *et al.*, 2002 |
| PTB (serum) | EIA | 30 kDa | 87 | 87 | Stavri *et al.*, 2003 |
| PTB (serum) | ELISA | 60 kDa | 94 | 92 | Walid Ben-selma *et al.*, 2010 |
| PTB (serum) | ELISA | 60 kDa | 75 | 92 | Gupta, S. *et al.*, 1997 |
| PTB (serum) | ELISA | RD1 and RD2, ESAT6, CFP10, CFP-21 | 90 | 90 | Mamta Kalra *et al.*, 2009 |
| PTB (serum) | ELISA, WB | MPT-32, 81 kDa, GicB | 68 | 90 | Krishna K. Singh *et al.*, 2003 |
| PTB (serum) | EIA | P-90 | 69 | 64 | Marcus B. Conde *et al.*, 2003 |
| PTB (serum) | ELISA | ES-31 kDa | 72 | 76 | Swati Banerjee *et al.* |
| PTB (serum) | ELISA | 38 kDa, MTB48, CFP10/ESAT6 | 73 | 77 | Xueqiong Wu *et al.*, 2009 |

**Table 3.** Emerging new TB diagnostics test[5]

| Diagnostic level | List of diagnostic test | Test category |
|---|---|---|
| Reference level | Liquid culture | Endorsed by WHO |
| | LPA for MDR-TB | |
| | Non-commercial culture and DST (MODS, NRA, CRI) | |
| | LPA for XDR-TB | Commercialized, technologies |
| | LPA for MDR-TB 2nd generation | |
| Intermediate level | 2-specimen approaches | Endorsed by WHO |
| | LED microscopy | |
| | Xpert MTB/RIF | |
| | Manual NAAT | Commercialized, technologies |
| | Rapid colorimetric DST | Technologies at feasibility stage |
| | Xpert 2nd generation | Technologies at early stages of development |
| Peripheral level | VOC detection | Technologies at early stages of development |
| | Enzymatic detection | |
| | Ag and Ab detection | |
| | NAAT 2nd generation | |

infection. Laboratories relying solely on antibody detection in early stages may miss this diagnosis. Similarly, if a patient reaches a laboratory for diagnosis after 4–5 months of infection, antibody detection may be more useful as the chances of getting higher antigens will be less due to masking of antigen levels by circulating antibodies present in the body. Using several antigens (as different antigens are produced at different stages), detection of antigens and antibodies will improve the sensitivity and specificity of the test. Table 2 represents the sensitivity and specificity of various serological tests used for the diagnosis of TB infection. As such tests can easily be performed by laboratory personnel, without need of any sophisticated instrument, they can be used in screening of TB where other tests are negative but clinical suspicion remains.

Biochemical test such as estimation of adenosine deaminase (ADA) is also used widely for diagnosis of PTB and EPTB[25]. ADA is not a very specific marker but has been found to give reliable results in EPTB cases, especially in pleural TB[26]. Moreover, the cost of ADA is very low (less than 100 INR) and can be a good option for preliminary screening of TB cases followed by serological, molecular and other tests for more definitive diagnosis. TST based on hypertensive immune response to purified protein of MTB has been in use for the past many years in diagnosis of TB (latent and active). However due to many limitations of TST, such as cross reactivity in individuals vaccinated with BCG and having infections not related to MTB, it is not recommended in TB diagnosis[27,28]. T cell test such as IGRAs based on interferon-γ secreted by T cells in response to antigens such as early secretary antigen target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) encoded in the region of difference 1 (RD-1) of MTBC, has been developed in recent times[29]. However clinical utility of IGRA in evaluation of patients with suspected or active TB still remains poorly defined.

## New diagnostics tests

Progress has been made over the past decade in improving existing TB diagnostics and developing new technologies, some of which have been endorsed by WHO (Table 3). Advent of newer techniques such as fluorescent microscopy with much cheaper fluorescence microscopes light emitting diodes (LEDs) has increased sensitivity by at least 10% (ref. 30), compared to conventional Ziehl-Nelson staining. Automated liquid culturing systems like BacT/ALERT 3D (BioMérieux, France) have been effective both with respect to culturing and providing confirmatory results[31]. These culturing systems are now regarded as modern gold standards and have been useful in cases where such methods cannot be used. Other alternatives, such as microscopically observed drug susceptibility (MODS)[32] and the nitrate reductase assay were also recently endorsed by WHO to overcome the cost and other problems related to liquid culture system.

Line probe assays such as GenoType MTBDR*plus* assay (Hain Life science GmbH, Nehren, Germany) and GenoType MTBDRsl assay are important tools developed for detecting drug resistance in smear-positive specimens. NAAT such as Gene Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is also a most promising development in TB diagnostics concerning MTB detection and its drug resistance[33]. Despite the advances in TB diagnostics, availability of such tests in developing nations, particularly in high endemic zones, has remained a dream. Due to this, many regions in developing world with limited

**Table 4.** BCG efficacy trials reported around the world with protective efficacy

| Type of study | Strain of BCG | Country | Protective efficacy | Reference |
|---|---|---|---|---|
| Clinical trial | Phipps | Native Americans | 100% | Stein, S. C. and Aronson, J. D., 1953 |
| Clinical trial | Copenhagen | England | 100% | WHO report, 1972 |
| Clinical trial | Park | Puetro Rico | 40% | Comstock, G. W. *et al*., 1974 |
| Case controlled | Mixed | Argentina | 90% | Miceli, I. *et al*., 1988 |
| Case controlled | Mareau | Belo Horizonte (Brazil) | 90% | Camargos, P. A. M. *et al*., 1988 |
| Case controlled | Japan and Pasteur | Indonesia | 70% | Putrali, J. *et al*., 1983 |
| Case controlled | Glaxo | England | 70% | Canetti, G. *et al*., 1972 |
| Clinical trial | Copenhagen | England | 70% | WHO report, 1972 |
| Clinical trial | Phipps | Native Americans | 70% | Stein, S. C. and Aronson, J. D., 1953 |
| Clinical trial | Park | Puetro Rico | 30% | Comstock, G. W. *et al*., 1974 |
| Clinical trial | Tice | Georgia, USA | 10% | Comstock, G. W. *et al*., 1976 |
| Clinical trial | Madras | Mandanapalle, India | 20% | Frimodt-Moller, J. *et al*., 1973 |
| Clinical trial | Copenhagen and Pasteur | Chingleput, India | 0% | Tripathy, S. P. *et al*., 1987 |
| Case controlled | Glaxo | England | 80% | Rodrigues, L. C. *et al*., 1991 |
| Case controlled | Pasteur | Cameroon | 60% | Blin, P. *et al*., 1986 |
| Case controlled | Glaxo | Kenya | 30% | Orege, P. A. *et al*., 1993 |
| Cohort | Glaxo | Karonga, Malawi | 0% | Ponnighaus, J. M. *et al*., 1992 |
| Randomized trial | Montreal | Canada | 80% | Ferguson and Simes, 1949 |
| Randomized trial | Glaxo | South Africa | 37% | Coetzee and Berjak, 1968 |
| Clinical trial | Moscow | Pune, India | Less | Deshpande, N. S. and Deshpande, S. V., 1995 |

resources still rely on conventional methods of mycobacterial detection. Thus development of improved diagnostics with focus on cost and availability of such tests in rural and remote settings is essential with respect to proper diagnosis and treatment to be initiated. Recently simpler molecular diagnostics test such as such Loop Mediated Isothermal Amplification (LAMP) has been evaluated in our laboratory. Comparative experiments showed that the LAMP assay is a rapid, sensitive and specific method for detection of TBM infection as compared to conventional nested-PCR assay and can be effectively set up in remote settings, with minimum cost[34,35].

## TB drugs

ATT started way back in 1944 and was generally used for immunotherapy involving drugs such as streptomycin and para-aminosalicylic acid (PAS). With discovery of new drugs in later years, combined drug therapy involving a combination of four drugs namely isoniazide (INH), rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA) was used. The existing drug regime can cure TB cases if patient is diagnosed very early and started on ATT. However owing to poverty, many people are unable to afford the current TB drugs. DOTS scheme was developed by WHO to avoid this problem but people still do not have access to these medicines. With lack of adequate facilities, many people fail to complete the treatment of 6–9 months. From our personal observation, it was noted that a majority of TB patients who were diagnosed and started on ATT do not come for follow up. Apart from this, there are some cases reported where people with adequate and timely treatment sometimes fail to respond to these drugs. Absorption of drugs, variable host response, nutrition, strain variation and co-infection with HIV are some causes responsible for failure of TB drugs. Some reports suggest that use of immunomodulators with TB drugs might be more useful than TB drugs alone, but this preliminary study needs extensive research and further evaluation to overcome the mentioned limitations[36,37].

## TB vaccines

A vaccine against TB was developed by Albert Calmette and Camille Guerin in 1921, using a live attenuated strain of *M. bovis*, Bacillus Calmette–Guerin (BCG). To date BCG remains the only available vaccine for protection against TB and is generally administered as single dose on deltoid region of arm few days after birth[38]. BCG vaccination was introduced in India in 1948 and in 1974 was included in expanded programme of immunization protocol with estimated worldwide coverage of about 85% (ref. 39). Despite its vast coverage, BCG has failed to reduce the immense burden of TB. Various reports suggest that although BCG provides substantial protection against meningeal and milliary TB in children, its protection rate in adult pulmonary disease is highly variable[40]. Comparison by a meta-analysis of various controlled trials revealed that the average protective efficacy of BCG in adults reaches 50% with an efficacy range from 0% to 80% protection[41] (Table 4). Therefore, the most prevalent form of TB in adult (i.e. LTBI) cannot be prevented by BCG satisfactorily.

In India, two major BCG vaccination trials carried out in Chingleput and Chennai reported that BCG did not

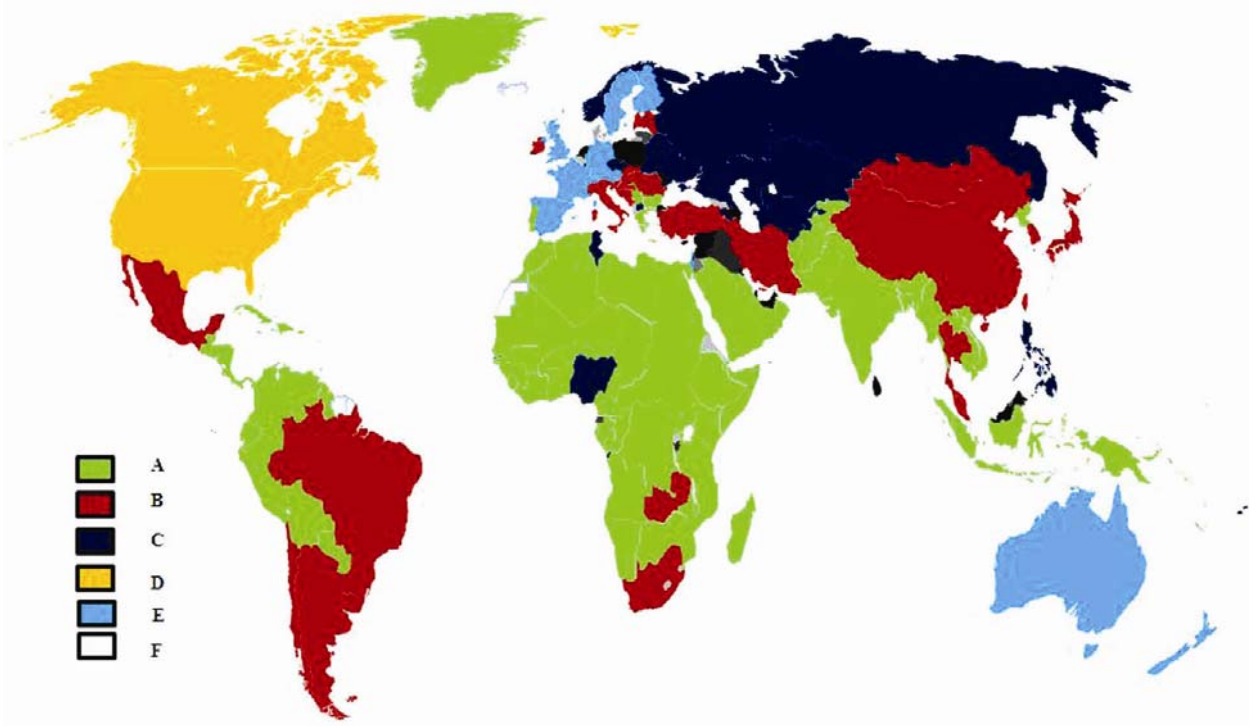


**Figure 4.** Global BCG vaccination policies. A, Countries who have adopted universal programme of BCG immunization and currently recommend a single dose of BCG. B, Countries who had earlier recommended but currently have ceased booster immunization of BCG. C, Countries promoting repeat vaccination of BCG. D, Countries who do not recommend BCG immunization. E, Countries who had earlier adopted universal programme of BCG and currently do not use it. F, Countries in which BCG vaccination status is unknown.

offer any protection against adult forms of bacillary PTB (Table 4). A number of reasons have been postulated for variable efficacy of BCG[42,43]. Currently WHO recommends the use of only a single dose of BCG vaccine based on lack of evidence supporting booster dose. However, due to variable protective efficacy of BCG, various countries have adopted different vaccination policies. Many countries such as India and United Kingdom have adopted universal programme of immunization. Figure 4 depicts global BCG vaccination policies adopted worldwide. In some countries such as USA and Canada, BCG is recommended only in persons with high risk of TB infection. Sixteen countries continue to give an additional BCG vaccination after the initial BCG, known as a booster vaccination and countries such as Kazakhstan, Belarus, Uzbekistan and Turkmenistan continue to recommend three BCG vaccinations in age group of 12–15 years[44].

## Current strategies in TB vaccine development

Recent advance in TB immunology has led to the development of improved molecules that can be used as a reliable alternative to BCG. According to the Global Plan to stop TB, 2006–2015, 'Effective TB vaccines will be an essential component of any strategy to eliminate TB by 2050'[45]. Many of the developed molecules have even advanced into Phases II and III clinical trials (Table 5). Current research in TB vaccinology includes development of subunit vaccines, DNA-based vaccines, recombinant vaccines which can be used in prime boost regimes based on boosting existing immune response due to BCG. Our lab has initiated some pilot studies on the use of prime boost regimes in improving the efficacy of BCG. Based on *in vitro* and animal studies our results suggest that use of BCG along with novel antigens such as Ag85B of MTB in heterologous regimes improves its efficacy. Recently new molecules such as peptide-based vaccine have been evaluated as promising vaccine candidates and alternative to antigen-based vaccine[46,47]. However, despite considerable research, availability of new and improved vaccines better than BCG has remained a distant dream. There are enough evidences and a dozen of candidate vaccines which have shown promising results in animal studies and initial clinical trials, but have not managed to surpass BCG as the universal vaccine. This suggests that revision on BCG policies in high TB burden countries is essential. As many of the vaccines are developed as pre-exposure and post-exposure vaccine, ability of particular candidate molecules to boost BCG-induced immune response and provide protection against TB remains doubtful in different study population. Another difficulty with the use of new vaccine is its potential to

**Table 5.** Current TB vaccine candidates in clinical trials

| Molecule | Type | Strategy | Antigens | Stage | Nature | Source |
|---|---|---|---|---|---|---|
| AERAS402/ Crucell Ad35 | Viral Vector | Prime-boost | Ag85A (Rv3804c) | Phase II b | Prophylactic | Crucell N.V/Aeras |
| RU TI | Fragmented MTB | Immunotherapeutic | Multi-Antigenic | Phase II | Therapeutic | Archivel Farma |
| Hybrid 1 | Recombinant protein with IC-31/CAF01 | Prime-boost | ESAT-6 (Rv3785c) fused to Ag85B | Phases I and IIa | Prophylactic | SSI/Intercell/TBVI |
| GSK M72 | Recombinant protein | Prime-boost | Rv1196 fused to an inactive serine protease Rv0125 with AS01 adjuvant | Phases I and IIa | Prophylactic | GSK/Aeras |
| MVA85A/ AERAS-485 | Viral Vector | Prime-boost | Ag85A (Rv3804c) Mtb32a (Rv0125) | Phase II b | Prophylactic | Oxford/Isis/Aeras/ Emergent |
| HyVac 4/ AERAS404 | Recombinant protein | Prime-boost | Ag85B fused to Mtb10.4 with adjuvant IC31 | Phase I | Prophylactic | Sanofi Pasteur/SSI/ Intercell/Aeras |
| VPM-X | Recombinant live | Prime | Native protein of BCG | Phase Ib | Prophylactic | Max Plank/VPM/ TBVI |
| Hybrid 56 | Recombinant protein with IC-31/CAF01 | Prime-boost | ESAT 6 (Rv3785) $R2660^{L}$ fused to Ag85B | Phase I | Prophylactic | SSI |
| Ad5Ag85A | Viral Vector | Prime-boost | Ag85A (Rv3804c) | Phase I | Prophylactic | CanSino Biotechnology Inc./Aeras |
| Mtb72F/AS01E | Recombinant protein with ASO1 Adjuvant | Prime-boost | Mtb39a (Rv1196) | Phase II b | Prophylactic | Aeras |
| ID93/GLAS E | Recombinant fusion poly protein | Prime-boost | Rv3619, Rv1813c, Rv3620c, Rv2608 | Phase I | Prophylactic | Infectious disease Research Institute |
| MOD-901 | Heated inactive *M. vaccae* | Prime | Poly antigenic | Phase III | Therapeutic | – |

mount an immune response in all sections of population rather than a particular one as those which are assessed in clinical trials. The unavailability of new vaccine along with unrevised vaccination policies in developing countries like India has led to emergence of increasing burden of TB, which is expected to surpass its present figure in coming years.

## Comments

The major problem in the underdeveloped and developing countries has been paucity of proper well-planned epidemiological studies on various aspects of TB. Data of any significance hardly is available on 'Latent Tuberculosis'. It must be realized that for proper planning and execution of any health programme, particularly in a chronic disease like TB, proper data is important. Even though, in our country, it is claimed by major Government organizations that incidence and prevalence of TB is coming down, the ground reality remains different, as cases of MDR are on global rise. To bring this disease under control we need to evolve new and rapid strategies for early diagnosis and properly monitored treatment. Revision in existing vaccination protocol is urgently needed to improve waning efficacy of BCG vaccine. We have to seriously consider even isolation of active 'open' cases of TB. We will have to tackle predisposing factors like hygiene, nutrition, control of disease like HIV/AIDS to achieve our goal in controlling TB infection.


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