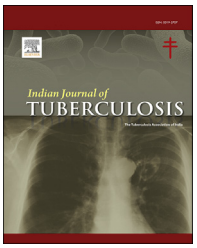
Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Review article

# A systemic review on tuberculosis

*Arvind Natarajan* [a], *P.M. Beena* [a], *Anushka V. Devnikar* [b], *Sagar Mali* [c,*]

[a] *Department of Microbiology, Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, India*

[b] *Department of Microbiology, S Nijalingappa Medical College, Bagalkot, India*

[c] *SDM Narayanaya Heart Centre, Sri Dharmasthala Manjunatheshwara Medical College, Sri Dharmasthala Manjunatheshwara University, Dharwad, India*

ARTICLE INFO

*Article history:*
Received 3 December 2018
Received in revised form
7 September 2019
Accepted 18 February 2020
Available online xxx

*Keywords:*
Tuberculosis pathogenesis
Immunology
Tuberculosis diagnosis
Tuberculosis treatment

ABSTRACT

Tuberculosis (TB), which is caused by bacteria of the Mycobacterium tuberculosis complex, is one of the oldest diseases known to affect humans and a major cause of death worldwide. Tuberculosis continues to be a huge peril disease against the human population and according to WHO, tuberculosis is a major killer of the human population after HIV/AIDS. Tuberculosis is highly prevalent among the low socioeconomic section of the population and marginalized sections of the community. In India, National strategic plan (2017–2025) has a national goal of elimination of tuberculosis by 2025. It requires increased awareness and understanding of Tuberculosis. In this review article history, taxonomy, epidemiology, histology, immunology, pathogenesis and clinical features of both pulmonary tuberculosis (PTB) and extra-pulmonary tuberculosis (EPTB) has been discussed. A great length of detailed information regarding diagnostic modalities has been explained along with diagnostic algorithm for PTB and EPTB. Treatment regimen for sensitive, drug resistant and extensive drug resistant tuberculosis has been summarized along with newer drugs recommended for multi drug resistant tuberculosis. This review article has been written after extensive literature study in view of better understanding and to increase awareness regarding tuberculosis, as a sincere effort that will help eliminate tuberculosis off the face of the earth in near future.



## 1. Introduction

> "*A dread disease in which the struggle between soul and body is so gradual, quiet and solemn and the result so sure that day by day and grain by grain, the mortal part wastes and withers away. A disease … which sometimes moves in giant strides and sometimes at a tardy sluggish pace, but, slow or quick, is ever sure and certain …*"
>
> Charles Dickens: Nicholas Nickleby.

Till date the words of Charles Dickens are true. Tuberculosis; A scourge of the mankind from time immemorial, the dread disease was called consumption in Dickens time had a profound social and economical effect on human existence

* *Corresponding author.*
E-mail address: sagar5838@gmail.com (S. Mali).

https://doi.org/10.1016/j.ijtb.2020.02.005



Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

worldwide.[1] Mankind has seen changing face of tuberculosis (TB): from an incurable disease to the curable one. With the emergence of HIV/AIDS epidemic (1981); the cursed and deadly co-infection of HIV and TB resulted in a global resurgence of TB.[2] In the early 1990's, a drug resistant TB strain caused an outbreak in New York, killing 80% of infected patients.[3] The HIV and TB co-infection, and spread of drug resistant TB has worsened the scenario to an extent that, TB has been declared a global emergency in 1993 by WHO.[2] The tuberculosis has varied presentation and it is divided into Pulmonary TB (PTB) and extrapulmonary TB (EPTB) based on clinical manifestation.[4] EPTB is defined as TB involving organs other than the lungs (e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, or meninges). If a patient with EPTB also has tubercular lesion in lung parenchyma, then the patient is categorized as pulmonary TB (e.g. military TB).[2] If the patient suffers from intra-thoracic mediastinal and/or hilar lymph node TB or TB pleural effusion without radiographic abnormalities in the lung is categorized as EPTB.[5] WHO estimates shows that globally there were 10.4 million cases of TB, in 2017, of which two thirds were in eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%).[6] EPTB constitutes about 15–20% of all TB cases. With HIV pandemic, the EPTB scenario is further complicated, as EPTB constitutes more than 50% of all cases of TB in HIV positive patients.[7] Due to its variety of presentation EPTB often poses a great difficulty in early diagnosis. It may present with constitutional symptoms such as fever, anorexia, weight loss, malaise and fatigue.[8] In India, the only presentation may be fever of unknown origin due to its remote infection site.[9]

Among the available modalities tuberculin skin test (TST), interferon gamma release assay (IGRA) and serological tests are not recommended in diagnosis of TB or initiation of Anti Tuberculosis Treatment (ATT).[10] Smear examination with sensitivity ranging from 10 to 37% and culture on Lowenstein Jensen (LJ) media with variable sensitivity ranging from 12 to 80% in different body fluids, also requiring 8 weeks of incubation for maximum sensitivity adversely affects the treatment plan by delaying it or subjecting patients to inappropriate empiric therapy.[11–14] This review article covers history, taxonomy, epidemiology, immunology, pathogenesis, clinical features of pulmonary TB and EPTB, all available diagnostic modalities followed by diagnostic algorithm and treatment to help understand tuberculosis.

## 2. History

TB or illnesses resembling TB have been described from different civilization since ancient times. The earliest such description can be found in Vedas, where TB was referred to as Yakshma meaning wasting disease. Greek, Chinese and Arabic literature also describes TB like disease.[2] Mycobacterium exists on earth since last 150 million years. Typical tubercular vertebral lesions were seen in mummies from the Egyptian pre-dynastic era and Peruvian pre-Colombian era. The first weak evidence of TB in humans is from a bone lesion found in a 500 thousand year old skull in Turkey. Human TB detection using PCR sequencing in a Neolithic infant and women from 9 thousand year old settlement in the Eastern Mediterranean is the oldest strong evidence available. Galen (131–201) first suspected that TB could be contagious. It took many centuries until Girolamo Fracastorius (1483–1553) showed that some diseases could be transmitted through 'particles' by direct or indirect contact between humans. Thomas Willis (1621–1675) first described miliary TB. Calmette extracted a protein (tuberculin) from large cultures of the bacillus and first used for therapy known as 'tuberculinisation', which failed as treatment for TB. The Tuberculin was also used for intradermal skin test which was described by Charles Mantoux & used in the diagnosis of TB. Later this intradermal skin test was named after Charles Mantoux and is known as Mantoux test.[15] Benjamin Marten (1690–1752) hypothesized that TB is caused by 'wonderfully minute living creatures' in his theory of 'contagious living fluid'. It was Jean Antoine Villemin (1827–1892), a French army doctor who successfully demonstrated the transmission of TB from humans to animals and from animals to animals. In 1834, Johann Lukas Schonlein proposed the name 'Tuberculosis' which is derived from Latin word 'tubercula' meaning 'a small lump' seen in all forms of the disease.[15] On 24th March 1882, Robert Koch announced in the meeting of the Berlin Society of Physiology that he had discovered causative agent responsible for pulmonary TB and named it as 'tuberkel virus' in his paper published 2 weeks later. First innovative decision of staining tuberculosis bacilli and second innovative decision of culturing it on solidified cow or sheep serum gave Robert Koch the Nobel prize of medicine in 1905. Leon Charles Albert Calmette (1863–1933) and Camille Guerin (1872–1961) developed vaccine against TB by sub-culturing *Mycobacterium bovis* for more than 200 times in the Guinea pig model between 1908–1921.[2,15] Arvid Wallgren, a professor from Royal Caroline medical institute, Sweden described clinical manifestations of tuberculous infection in an article titled 'The timetable of Tuberculosis' which helped in better understanding course of TB illness.[16] The effective treatment for TB became a reality after the discovery of antitubercular drugs like Streptomycin, Para-amino salicylic acid (PAS) and isoniazid by the mid-1940s. By late 1970 it was believed that TB may no longer be a public health problem in the developed world. But the emergence of Acquired Immune Deficiency Syndrome (AIDS) in the early 1980s has ended this optimism and led to the resurgence of TB worldwide.[2]

## 3. Taxonomy and description of the genus

*Mycobacterium tuberculosis* belongs to

ORDER- Actinomycetales
CLASS- Actinomycetes
FAMILY- Mycobacteriaceae
GENUS- Mycobacterium

Genera closely related to *Mycobacterium* are Gordomia, Tsukamurella, Nocardia and Rhodococcus.

Features of genus Mycobacterium are summarized in Table 1.

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

**Table 1 – Salient features of *Mycobacterium* genus.[17,18]**

| | Features |
|---|---|
| Mycobacteria | aerobic, non-spore forming, non-motile |
| Shape | slightly curved or straight rods |
| Size | 0.2–0.6 μm by 1–10 μm |
| Colony morphology | varies from species to species, ranging from rough to smooth and from non-pigmented to pigmented (carotenoid pigment) |
| Cell wall | N-acetyl muramic acid<br>High content of Mycolic acid (70–90 carbon atoms)-renders acid fastness |
| DNA | High G + C content (61–71 mol %) |
| Generation time | Slow- ranging from 20 hours to 36 hours for Mycobacterium Tuberculosis |

## 4. Epidemiology

*M. tuberculosis* bacilli have infected nearly 1/3rd of the world's population with 10% lifetime risk of developing TB disease.[19] Globally 10.4 million cases of TB reported in 2017, accounting to 133 cases/1,00,000 population, of which 90% of cases were adults (aged ≥15 years), 64% were male, 9% were people living with HIV (72% of them in Africa) [Fig. 1]. An estimated 558 000 new cases (range- 483 000–639 000) of Rifampicin resistant TB (RR-TB), of which almost half were in three countries: India (24%), China (13%) and the Russian Federation (10%).[6] Among 0.8 million new EPTB cases reported worldwide (2013), maximum cases were from India accounting for 0.35 million cases.[20] In India, according to Revised National Tuberculosis Control Programme (RNTCP) data, the prevalence of EPTB is 50% in HIV infected patients and 15–20% in non-HIV patients. The distribution of EPTB was in lymph node 47%, pleural cavity 30%, abdomen 10%, bones and joints 8%, CNS 2% and others 3%.[21] Between 2000 and 2017, TB mortality rate reduction is 42%. TB incidence has fallen by an average of 2% per year and case fatality rate of 16% in 2017, down from 23% in 2000.[6] In 30 high burden countries, India has managed to reduce the prevalence rate by 50% as set by Stop TB Partnership Programme. Drug resistant TB has been reported from early days of introduction of ART, but multidrug-resistant tuberculosis (MDR-TB) and more recently extensively drug resistant tuberculosis (XDR-TB) posing a threat to TB control program globally.

## 5. Pathogenesis

The majority of droplet nuclei containing MTB from infectious patients are trapped in upper airway and expelled by ciliated mucosal cells: only a fraction reaches alveoli. The mycobacteria then bind to cell surface of alveolar macrophages through complement receptors, mannose receptor or type A scavenger receptor. Following phagocytosis, mycobacteria reduce acidity in phagosome and a cell wall component (i.e. lipoarabinomannan) impairs Ca+/calmodulin pathway thus inhibiting phagosome-lysosome fusion. Following successful arrest of phagosome maturation, the multiplication of bacilli begins and the macrophage eventually ruptures to release its bacilli, which are taken up by macrophages and continues infection cycle further expanding the spread.[22] During primary infection, MTB bacilli undergo hematogenous and lymphatic dissemination involving hilar and mediastinal lymph nodes forming primary Ghon's complex. Eventually bacilli enter blood stream and reach various organs. This lympho-hematogenous dissemination results in extrapulmonary tuberculosis during primary infection or later in life during reactivation of disease.[23] EPTB can involve any site in the body & the most common site is lymph node. However pleural, neurological, synovial, pericardial, abdominal, genitourinary involvement has been described.

### a. Tubercular Lymphadenitis

From ancient times, lymph node TB has been called Scrofula or King's evil. It constitutes nearly 35% of EPTB cases. Cervical lymphadenitis is the most common and reported in 60–90% of tuberculous lymphadenitis cases. Involvement of cervical lymph node is due to spread of bacilli from primary focus of infection in Ghon's complex or from tonsils, adenoids, sinonasal/osteomyelitis of the ethmoid bone. Initially, MTB bacilli multiply in lymph node causing marked hyperemia, swelling, necrosis and caseation of involved lymph node. The inflammation, progressive swelling and matting of other nodes around, resulting in adhesion to adjacent skin and

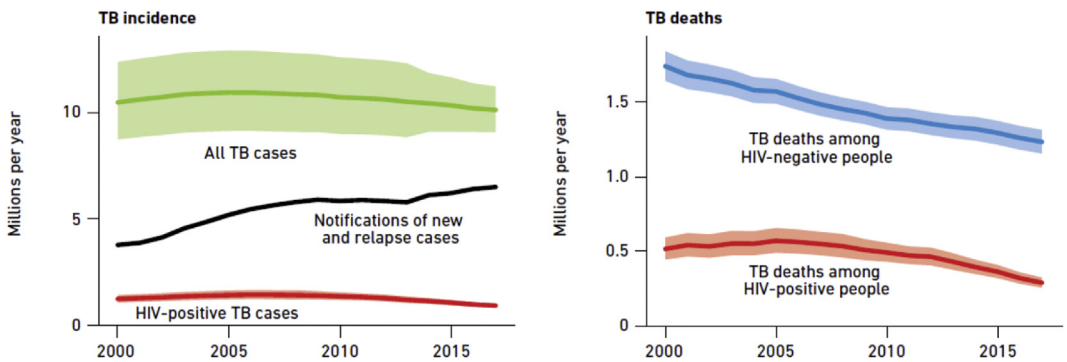



**Fig. 1 – Shows an estimated number of incident TB cases and TB deaths (in millions) from 2000 to 2017.[6]**

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

rupture into surrounding tissue or through skin forming sinuses. Mediastinal lymphadenitis can compress major blood vessels, phrenic nerve or recurrent laryngeal nerve or cause erosion of bronchus which is commonly seen in children.

Peripheral tuberculosis of lymph nodes is classified by Jones and Campbell into-

STAGE I:-Enlarged, firm, motile discrete nodes.
STAGE II:-Large rubbery nodes fixed to surrounding tissue.
STAGE III:-Central softening due to abscess formation.
STAGE IV:-Collar stud abscess formation.
STAGE V:-Sinus tract formation.[24]

b. **Pleural TB**

The incidence of pleural TB is as high as 30% of all EPTB cases in high burden countries. The patients usually presents with acute febrile illness with nonproductive cough and pleuritic chest pain; associated with night sweats, chills, weakness, dyspnea, weight loss. The pathogenesis in pleural TB is presumed to be due to delayed hypersensitivity rather than direct infection of pleural space. This space is infected from initial lung parenchymal lesions and results in immunological response predominated by neutrophils (first 24 hours). This is followed by lymphocyte driven immune response forming pleural granuloma formation and release of Adenosine Deaminase (ADA). Neutrophils remain the first line of defense for first 24 hours, followed by macrophages which peak at 96 hours and then by lymphocytes. A strong T-helper type-1 (Th 1) response is necessary to contain MTB. Activated CD3+ and CD4+ Th1 cells release interferon $\gamma$ (IFN- $\gamma$) thus activating macrophages to kill MTB. The Th1 immunity in pleural TB is confirmed by the high levels of IFN- $\gamma$, interleukin-12 (IL-12) and elevated helper T cells in pleural fluid as compared to serum/peripheral blood. The delayed hypersensitivity reaction to mycobacterial antigens affects pleura and increases the permeability of pleural capillaries, and thereby increasing fluid in pleural cavity. The fluid is drained through openings in the parietal pleura called stomata. Since diffuse involvement of parietal pleura with TB and damage to or obstruction of stomata leads to accumulation of pleural fluid. Chronic TB empyema resolve leaving thickened, scarred and calcified pleura causing chronic chest pain, dyspnea and impaired lung function. Pleural fibrosis, a well-documented complication has been reported in 5–55% of pleural TB cases.[25,26]

c. **Abdominal TB**

The abdominal tuberculosis is diagnosed in 11% of patients with EPTB which was around 55–90% in era before effective ATT. The most common site of gastrointestinal tract involvement is the ileocecal region due to following reasons-

i. More lymphoid tissue (Peyer's patches)
ii. Increased physiological stasis
iii. Rate of fluid and electrolyte absorption is more
iv. Low digestive activity

Other sites of involvement in decreasing order are ascending colon, jejunum, appendix, duodenum, stomach, esophagus, sigmoid colon and rectum. Hepatobiliary, splenic and pancreatic TB are rare and associated with miliary tuberculosis; often diagnosed in immunocompromised patients. MTB bacilli gain entry to abdominal organs by two routes and cause disease due to reactivation of dormant focus. As a result of hematogenous spread from primary lung infection in children and as a part of miliary TB. Through ingestion of contaminated food and milk which infect Peyer's patches and are transported to mesenteric lymph node, where they remain dormant.[27,28]

d. **Central Nervous System (CNS) TB**

It is serious and often fatal form of EPTB, predominantly affecting young children. CNS TB is difficult to diagnose. It presents in 2 major forms

i. TB meningitis- 0.5–1% of all TB cases
ii. Intra-cranial tuberculoma-accounting up to 40% of brain tumors

MTB bacilli reach CNS during dissemination that occurs in active pulmonary disease. These bacilli cross physiological Blood Brain Barrier (BBB) via infected monocytes/neutrophils and cause a caseating focus in brain parenchyma or meninges. These foci are termed as 'Rich foci'. Later, these foci rupture in subarachnoid space triggering inflammatory T cell response with elevated levels of INF $\gamma$ and TNF-$\alpha$ in CSF. The subsequent inflammation leads to production of inflammatory infiltrates which obstructs CSF outflow causing hydrocephalus and vasculitis leads to infarction, causing potentially irreparable neurological damage.[29]

e. **Bone and Joint TB**

It accounts for 10–15% of all EPTB cases. It arises from reactivation of dormant MTB bacilli lodged in any bone (spine or large joints) during bacteremia of primary lung infection. These bacilli have affinity for spine and large joints because of their rich vascular supply. An extension of initial infection focus from the bone to the joint results in tuberculous arthritis. Rarely the bacilli can reach spine from the lung along the Batson paravertebral venous plexus or by lymphatic drainage to the paraaortic lymph nodes. Non tuberculous mycobacteria (NTM) have been reported to cause osteoarticular TB following a traumatic injury or during surgical procedure like joint arthroplasty. NTM bone infection in patients with AIDS or transplant recipients occurs through hematogenous dissemination. In recent years M. bovis skeletal infections have been reported in individuals who receive intravesical BCG vaccine therapy.[30]

f. **Genito-urinary TB (GUTB)**

It accounts for 15% of all EPTB cases and 3–4% of all PTB cases. Its occurrence is 20 times more in kidney transplant recipients than in general population.[12] After hematogenous spread of bacilli from active site of infection (usually lungs), bacilli gets lodged in kidney (most common site of GUTB) and form metastatic lesions (tubercles). These foci of infection

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

may heal spontaneously/due to treatment, enlarge and rupture into nephrons or remain dormant for many years. Usually the spread of infection is descending from kidney to other genito-urinary organs. It develops between 2nd and 4th decades of life; usually 5–25 years of inactivity after primary lung infection.[31]

g. **Miliary TB**

Miliary TB account for less than 2% of all tuberculosis cases and up to 20% of all EPTB cases among immunocompetent adults, however the autopsy studies have shown military TB ranges between 0.3% and 13.3%. TH2 response plays a central role in immunopathogenesis of military TB. It inhibits protective response such as granuloma formation and fencing of the disease activity at the site of infection. The production of interleukin 4 (IL-4) during TH2 response, downregulates nitric oxide synthase (NOS), tall like receptor 2 and macrophage activation; thus sabotaging protective response of TH1 cells. This process favors dissemination of MTB.[32]

## 6. Histology

Any site of infection involved in PTB or EPTB has pathognomonic lesions known as tubercles. This is characteristic granulomatous inflammatory reaction against MTB bacilli from host's cell mediated immunity. These tubercles are microscopic to begin with and coalesce to become macroscopically visible granulomas. The granulomas contain MTB bacilli within macrophages, fibrin rich alveolar exudate, lymphocytes and multinucleated giant cells which are enclosed within fibroblastic rim. These granulomas formed are both caseating and non-caseating granulomas[33] (Fig. 2A,B and C).

## 7. Immunology

Robert Koch (1880) demonstrated delayed hypersensitivity reaction in Guinea pigs and later in humans using mycobacterial extracts. Seifert (1934) purified MTB extract which later become reference used as Purified Protein Derivative (PPD) in tuberculin test. In 1945 M. Chase demonstrated that immunity against MTB cannot be transferred to animals by immune serum but by transfer of CD4 T lymphocytes. It is now clear that protection against MTB is through mainly T lymphocyte activating the macrophages. Dendritic cells (DCs) present in proximal draining lymph nodes play a major role in priming naïve T cells. DC takes part in surveillance around airways, vessels and in the loose connective tissue. The mycobacterial specific lipoglycan lipoarabinomannan (LAM) binds to receptor present on DC to gain entry. The lipoid adjuvant of LAM activate antigen presenting cells (APC) through toll like

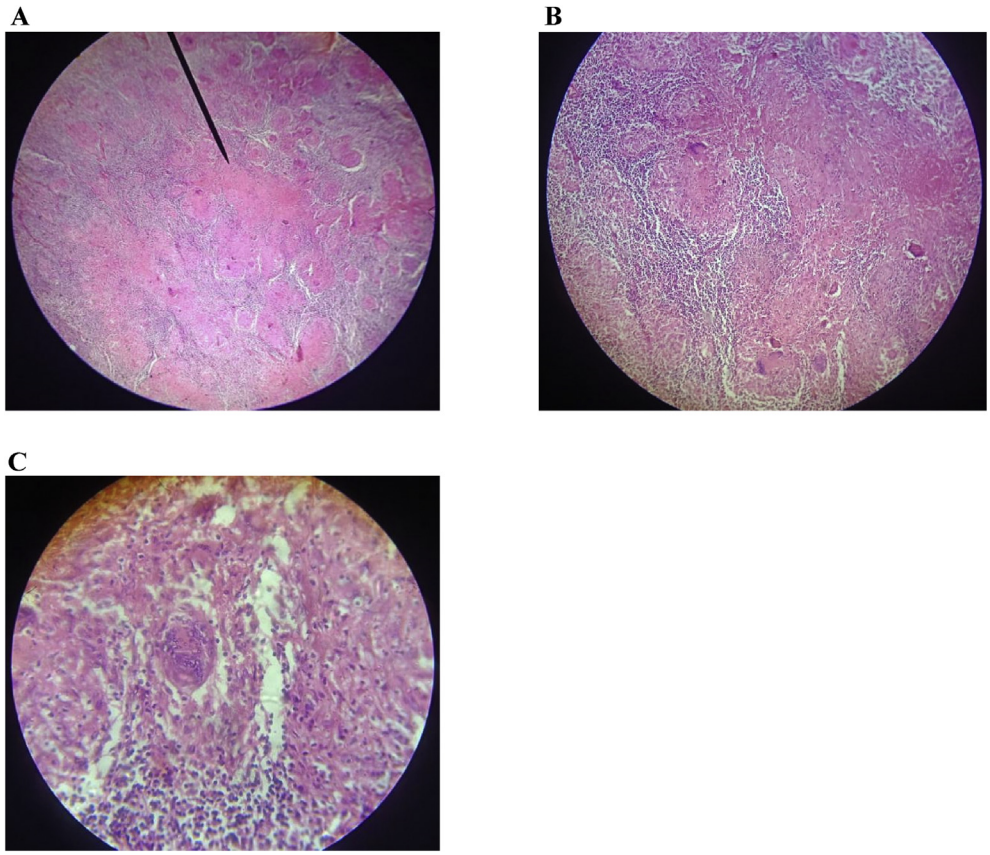



**Fig. 2 – (A) Caseous necrosis. (B) Granuloma surrounded by lymphocytes. (C) Multinucleated giant cells & lymphocytes in tubercular granuloma.**

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

receptor-2 (TLR-2). Both DC and APC ultimately prime T lymphocytes, after which memory CD4 and CD8 cells play central role in immune response against MTB. The activated CD4 and CD8 cells have ability to kill intercellular MTB through secretion of cytolytic molecules (e.g granulysin, perforin) and chemokines (e.g CCL5 which attracts infected macrophages). Natural killer cell (NK cells) acts as bactericidal against MTB which are part of innate immunity. NK cells do not require APCs for their activation and also improve function of γ δ T cells. The γ δ T cells are mycobactericidal and potent secretors of INF-γ like macrophages. The activated T lymphocytes release IFN γ, TNF- α, and Interleukin-2 activating other resting monocytes/macrophages. γ IFN up regulates production of TNF, toxic oxygen species and nitric oxide in macrophages. These lead to granuloma formation and effective containment of MTB inside granuloma.[34]

## 8. Clinical features

EPTB is less common when compared to PTB, thus less commonly encountered by clinicians and difficult to diagnose clinically.

1. **Miliary TB**

The clinical features are usually non-specific and may present with fever, weight loss, night sweats, anorexia and weakness. The physical findings in descending order are fever, wasting, hepatomegaly, pulmonary findings, lymphadenopathy and splenomegaly. A granuloma in retinal choroid is strong suggestive feature of disseminated TB.[32,35]

2. **TB Lymphadenitis**

It presents as painless swelling in cervical region (supraclavicular fossa). Usually the process is bilateral and with progression of disease, the lymph node fuse and become matted. The overlying skin gets inflamed, ultimately enlarged lymph node rupture through inflamed skin forming sinus tract. Intra thoracic adenopathy may cause atelectasis by compressing bronchi or bronchiectasis (common in children).[24,36]

3. **Pleural TB**

The presentation in tubercular pleurisy depends on number of bacteria infecting pleural space. If few MTB bacilli gain entry into pleural space, then it leads to hypersensitivity response causing pleural effusion. The process may resolve spontaneously or may lead to large effusion causing fever, pleuritic pain, dyspnea and weight loss. If large number of MTB bacilli gain entry from rupture of a cavity or the adjacent parenchymal fistula, then it leads to tubercular empyema. The presentation of pleural TB in HIV seropositive patients is chronic with additional symptoms like tachypnea, night sweats, fatigue, diarrhea and have more hepatomegaly, splenomegaly, lymphadenopathy as compared to seronegative patients.[26,35]

4. **Abdominal TB**

The clinical presentation depends on site of involvement as TB can affect any location from mouth to anus. The most common site of involvement is terminal ileum or caecum and manifest as pain abdomen, a palpable mass sometimes with weight loss, fever and loss of appetite. Tubercular peritonitis presents with classic doughy abdomen, ascites, pain abdomen and fever.

In esophageal TB, additional symptoms seen are dysphagia, odynophagia and retrosternal pain/discomfort. Patient also suffers from life threatening complications like broncho-esophageal fistula/hematemesis.

Gastric TB is rare because of acidic pH, few lymphoid tissues in mucosa and rapid gastric emptying. Duodenal TB presents with dyspepsia, duodenal obstruction and duodenal ulceration. Other reported complications are perforation, fistulae and obstruction jaundice. The common presenting feature in rectal TB is hematochezia followed by constitutional symptoms and complication. It may also present as anal fissure, fistulae or perirectal abscess.[28,35]

5. **CNS TB**

The most common manifestation of CNS TB are meningitis (95%), tuberculomas (2%) and abscess (1%). Clinical features include those related cranial nerve involvement as well as headache, vomiting, decreased level of consciousness, neck stiffness and in the absence of medical care coma and death.[35,36]

6. **Skeletal TB**

Pain is the most common presenting feature. The involved joints have limited motion of range with or without the presence of swelling. The patient may present with sinus tract.

Involvement of spine leads to chronic backache, fever and more than 50% of patient suffer from neurological symptom due to compression of spinal cord. Delayed diagnosis may further complicate the situation due to spinal deformity and severe, irreversible neurological sequelae like paraplegia.[30,35]

7. **Genito-urinary TB**

Patient usually presents with local symptoms like dysuria, hematuria, flank pain and increased frequency of micturition. In women, genital involvement presents with pelvic pain, menstrual irregularities and infertility whereas in men the most common presentation is scrotal swelling/mass with or without pain. Symptoms of prostatitis, orchitis or epididymitis may also occur depending on site of involvement.[31,35]

## 9. Lab diagnosis

Definitive diagnosis of tuberculosis involves demonstration of *M. tuberculosis* bacilli by microbiological, cytopathological or histopathological methods.[2] The classic laboratory approach to the diagnosis of mycobacterial infections involves the phenotypic characterization of colonies growing on Lowenstein–Jensen medium. The current recommendation is

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

that a combination of phenotypic and molecular assay are used for the rapid identification of mycobacteria, particularly for the identification of *M. tuberculosis*.[37] Accurate diagnosis of EPTB depends on the detection of mycobacteria by using direct and indirect approaches.[7] The tests used in the diagnosis of tuberculosis are listed in Table 2.

### 9.1. *Microscopy*

Mycobacteria are recovered from variety of pulmonary and extrapulmonary samples. At least 10 000 AFB should be present per ml of sample for them to be readily demonstrable in direct smears.[38] Microscopy is reliable, reproducible, inexpensive, an indicator of infectiousness, a comprehensive tool for diagnosis/monitoring progress/defining cure and even feasible in the remote or tribal places.[39]

WHO evaluation showed that the diagnostic accuracy of light – emitting diode (LED) microscopy is comparable to that of conventional fluorescence microscopy with much less expense.[7] RNTCP has provided light emitting diode based fluorescent microscope services in 200 medical colleges across India as a pilot study.[39]

#### 9.1.1. *Acid fast staining*

The cell wall of mycobacteria, because of their high lipid content, have the unique capability of binding the Fuchsin dye so that it is not removed (destained) by acid alcohol. The presence of acid fast bacilli (AFB) in the smear, combined with history of weight loss, fever, night sweats and radiological evidence of old pulmonary lesion helps in early diagnosis. Acid fast smears are also useful in monitoring response to treatment.

Types of acid-fast stains used are:

i. Carbol fuchsin stains: a mixture of fuchsin with phenol (carbolic acid)Ziehl-Neelsen (hot stain)Kinyoun (cold stain)
ii. Fluorochrome stain: Auramine O, with or without a second fluorochrome, rhodamine.[37]

1. **Ziehl-Neelsen (hot stain):-**

The reagents and dyes used are-

Carbol fuchsin-dissolve 3g of fuchsin in 10ml of 90–95% ethanol. Add 90ml of 5% aqueous solution of phenol.

Acid alcohol-add 3ml of concentrated HCL slowly to 97ml of 90–95% ethanol.

Methylene blue counterstain-dissolve 0.3g of methylene blue chloride in 100ml of distilled water.

Examine with 100× oil-immersion objective. Mycobacteria are stained red and the background light blue.

The heating process during staining helps to penetrate carbol fuchsin into the MTB bacilli. The smear is decolorized using 20% $H_2SO_4$ and counter stained with methylene blue. MTB bacilli resist decolourisations by 20% $H_2SO_4$ hence are known as AFB.[37]

Ziehl-Neelsen staining is reliable, reproducible and cost effective and also useful in monitoring response to anti TB treatment. Smear microscopy has low and variable sensitivity values (0–40%) and could not differentiate between *Mycobacterium tuberculosis* and nontuberculous mycobacteria.[40–42]

2. **Kinyoun (cold stain):-**

The reagents and dyes used are-

Carbol fuchsin-dissolve 4g of basic fuchsin in 20ml of 90–95% ethanol and then add 100ml of 9% aqueous solution of phenol (9g of phenol dissolved in 100ml of distilled water)

Acid alcohol-add 3ml of concentrated HCL slowly to 97ml of 90–95% ethanol.

Methylene blue counterstain-dissolve 0.3g methylene blue chloride in 100ml of distilled water.

Examine with 100× oil immersion objective.

Mycobacteria are stained red and the background light blue. This technique is cold stain technique because increased phenol concentration replaces heating step and helps in penetration of carbol fuchsin.[37]

3. **Auramine Fluorochrome:-**

The reagents and dyes used are-

Phenolic Auramine-dissolve 0.1g of Auramine O in 10ml of 90–95% ethanol and then add to a solution of 3g of phenol in 87ml of distilled water. Store the stain in a brown bottle.

Acid-alcohol- add 0.5ml of concentrated HCL to 100ml of 79% alcohol.

Potassium permanganate-dissolve 0.5g potassium permanganate in 100ml of distilled water.

**Table 2 – List of tests used in laboratory diagnosis.**

| Microscopy | Culture | Conventional methods | Molecular methods |
|---|---|---|---|
| Acid fast staining<br>i. Ziehl-Neelsen<br>ii. Kinyoun<br><br>Fluorochrome staining | Solid Media<br>a. Egg-based Media<br>b. Agar-based Media<br>c. Selective Media<br><br>Liquid Media<br>a. MGIT<br>b. BACTEC 460TB system<br>c. Automated Continuous Monitoring Systems<br><br>HPLC(for culture confirmation and speciation) | i. Rates of Growth<br>ii. Pigment production<br>iii. Niacin accumulation test<br>iv. Nitrate reduction test<br>v. Tween 80 Hydrolysis<br>vi. Catalase test<br>vii. Arylsulfatase activity<br>viii. Urease activity<br>ix. Pyrazinamidase<br>x. Iron uptake<br>xi. Growth on MacConckey<br>xii. Growth in 5% sodium chloride | 1. Nucleic acid probes<br>2. In situ hybridization<br>3. Nucleic acid amplification methods<br>4. Line probe assay<br>5. Transcription mediated amplification<br>6. DNA sequencing<br>7. Spoligotyping<br>8. DNA microarray analysis<br>9. CBNAAT<br>10. PBMC circ RNA detection |

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

Examine with 25× objective for scanning. The 40× objective is used to confirm any suspicious forms. Mycobacteria are stained yellow-orange against a dark background in fluorescent microscope.

The fluorochrome stain offers the advantage of greater sensitivity compared with the ZN stain, since a significantly larger area of the smear can be scanned thus reducing the time needed. Fluorescent microscopy increases sensitivity by 10% over ZN staining.[37]

RNTCP has supplied light emitting diode based fluorescent microscope (LEDFM) in 200 medical colleges across India to reduce the burden on laboratory technicians in high work load settings (>25 slides per day).[39]

### 9.2. *Culture*

Isolation of *M. tuberculosis* from clinical samples by culture is the 'gold standard' for a definitive diagnosis of TB. Culture methods are much more sensitive because fewer bacilli (10–100 bacilli/ml of concentrated material) can be detected and provides the necessary isolates for conventional drug susceptibility test and species identification. The sensitivity of culture for identification of M TB ranges between 0 and 80% in different extrapulmonary specimens.[9,43–45] The most commonly used solid media for culture of M TB is LJ media which usually takes 4–8 weeks for visible growth.[46]

#### 9.2.1. *Solid media*

1) **Egg-based Media**

It contains whole eggs or egg yolk, potato flour, salts and glycerol are solidified by inspissation. They have good buffer capacity and a long shelf life and support good growth of most of mycobacteria. Of the egg-based media, L-J medium is most commonly used in clinical laboratories.

2) **Agar-based Media**

In contrast to egg-containing media, agar based media are chemically better defined. Colonies may be observed in 10–12 days, in contrast to 18–24 days with egg-based media. Thinly poured 7H11 agar plates can grow micro colonies in 11 days and can be examined by focusing agar surface through the bottom of the plate at 10 × to 100× magnification. This method is used as an alternative to broth cultures. It can also be used for susceptibility testing.

3) **Selective Media**

The addition of antimicrobial agents may be helpful in eliminating growth of contaminating organisms. If a selective medium is used for a particular specimen, it should not be used alone but in conjunction with a non-selective agar or egg-based medium. LJ media is made selective by adding penicillin and nalidixic acid, this is called Gruft modification or it can be made selective with cycloheximide, lincomycin, and nalidixic acid. Mitchison selective 7H11 medium contains carbenicillin, polymyxin B, trimethoprim and amphotericin B.[18]

#### 9.2.2. *Liquid media*

Broth media can be used for early isolation of mycobacteria and also for subsequent subculturing. Middlebrook 7H9, BACTEC 12B and Dubos Tween albumin broth are commonly used liquid media. 7H9 broth is used as a basal media and Tween 80 acts as a surfactant which disperses clumps of mycobacteria resulting in homogenous growth. The liquid media are currently used in semi-automated and automated like BACTEC 460TB system, BACTEC MGIT 960 system, BACTEC 9000MB, ESP culture system 2 and MB/BacT ALERT 3D system, BACTEC MYCO/F lytic blood culture bottle.

1. **Mycobacteria Growth Indicator Tube (MGIT):-**

The MGIT contains a modified Middlebrook 7H9 broth in conjunction with a fluorescence-quenching-based oxygen sensor (silicon rubber impregnated with a ruthenium pentahydrate) to detect growth of mycobacteria. The presence of oxygen in the medium quenches the fluorescence of the sensor. As mycobacteria or other organisms grow in the broth, leads to depletion in the oxygen level and the indicator fluoresces brightly when illuminated with UV light at 365nm. The broth is enriched with 0.5ml of OADC (Oleic Acid, Bovine Albumin, Dextrose and Catalyse) and 0.1ml of PANTA antibiotic mixture (Polymixine B, Amphotericin B, Nalidixic Acid, Trimethoprim and Azlocillin). In BACTEC MGIT 960 system the tubes are continuously monitored by the instrument. Sensitivity and time to growth detection of the MGIT system are similar to those of the BACTEC 460TB system and have been superior to those obtained with solid media in clinical evaluation. But contamination rates are slightly higher for MGIT system than for BACTEC 460TB system.[18,47]

2. **BACTEC 460TB system:-**

It is a semi-automated system which uses 14C-labeled palmitic acid as carbon source in the medium and when metabolized by microorganisms to 14CO2, it is monitored by the instrument. The amount of $14CO_2$ and the rate at which the gas is produced are directly proportional to growth rate of the organism in the medium. The average detection time for smear positive specimen is 9–14 days in case of *M. tuberculosis* and less than 7 days for NTM. The positive vials can also be used for drug susceptibility testing. The disadvantages of this system include inability to observe colony morphology, difficulty in identifying mixed cultures, overgrowth by contaminants, cost and radioisotope disposal.[18]

3. **Automated Continuous Monitoring Systems:-**

The BACTEC 9000 MB system uses fluorescence-quenching-based oxygen sensor same as the MGIT system to detect growth. In ESP Culture System 2, growth is detected by monitoring pressure changes in the headspace above the broth medium in a sealed bottle resulting from gas production by microorganisms. The MB/BacT ALERT 3D system employs a colorimetric $CO_2$ sensor in each bottle and reflected light to monitor the presence and production of $CO_2$ dissolved in the culture media. As the microorganism grow, $CO_2$ is generated which diffuses through membrane to sensor and dissolves in

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

water present in the sensor causing accumulation of hydrogen ions.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H+ + HCO_3$$

The amount of $CO_2$ produced is proportional to the growth of microorganism in the media, as the $CO_2$ levels increase, the concentration of hydrogen ions increases, thus reducing the pH of sensor causing color change from dark green to light green or yellow.

A light emitting diode system projects light on the sensor and the reflected light is measured by photodetector. The color change of sensor increases reflectance units which is monitored and recorded by the instrument to determine the positive or negative result.[18]

BacT ALERT 3D system has been evaluated in many studies for rapid detection of growth and also for DST. A study by Carricajo A et al. reported mean detection time of M TB complex from pulmonary and different extrapulmonary samples were 22.8 days with LJ medium and 16.2 days with the BacT ALERT 3D system.[47] In study by Piersimoni et al. Mean detection time of M TB complex in smear positive samples by BacT ALERT 3D, B460 and LJ media was 11.5, 8.3 and 20.6 days respectively whereas in smear negative samples it was 19.9, 16.8 and 32.1 days respectively.[49]

These studies have reported that mean detection time taken by BacT ALERT 3D system was 16–18 days compared to 22–32 days by using LJ media for M TB complex. Thus BacT ALERT 3D system reduces detection time by 25%.[48–50] In another study by Moore WAJ et al. the median time to culture positive was 7 days, 13 days and 26 days for microscopic observation drug susceptibility culture, automated culture and LJ culture respectively.[51]

The BACTEC MYCO/F LYTIC culture bottle has lytic agent to release mycobacteria phagocytosed by white blood cells. The incubation and monitoring is similar to other BACTEC blood culture bottles. It can also be used to culture other bacteria and fungi present in blood stream.[37]

#### 9.2.3. *Gas-liquid and high-performance liquid chromatography (HPLC)*

Analysis of fatty acids by gas–liquid chromatography is a rapid and reliable method for the culture confirmation mycobacteria and also identification of the species. The method is based on development of profiles of mycolic acids, which vary from one species to another. This technology to speciate mycobacteria is available only in few reference laboratories.[37]

Denaturing HPLC is an alternative for other methods which utilizes a molecular probes like DNA sequence analysis (which is regarded as the gold standard for mutation detection), reverse line hybridization, single-strand conformation polymorphism, DNA macroarrays and real-time PCR. This technique is relatively inexpensive, same-day results can be obtained, potentially any mutation in the amplified fragment can be detected and it can be applied on a universal basis. Other studies have shown this method can be useful for detecting mutations on rpoB (RMP), katG (INH), pncA (pyrazinamide), rspL (streptomycin) and embB (ethambutol) gene.[52]

### 9.3. *Identification using conventional methods*[37]

i. **Rates of Growth**

A rate of growth, the time of recovery varies from media to media-the average time of recovery of mycobacteria on egg based media is about 21 days, but ranges from as short as 3–5 days to as long as 60 days depending on the species. The growth of micro colonies on 7H10 agar is detectable from 3 to 12 days. In a study the average time to detection of micro colonies of MTB was 11 days on middlebrook 7H11 agar, 16 days with MB/BacT bottles and 19.5 days with LJ media. Some mycobacterial species belonging to rapid grower group grows within 7 days on LJ media.

ii. **Pigment production**

Mycobacterium species have capability of producing colony pigmentation in the dark (scotochromogen) or only after exposure to light (photochromogen) doesn't finalize species identification but narrows possibilities. Even after exposure to light MTB fails produce pigment, beyond a light buff color.

**Non tuberculous mycobacteria**

Non tuberculous mycobacteria (NTM) has been classified on the basis of pigment production and rate of growth by Runyon-

GROUP I- Photochromogens
GROUP II- Scotochromogens
GROUP III- Nonchromogens
GROUP IV- Rapid growers

iii. **Niacin accumulation test**

All mycobacteria produce niacin, but only MTB and M. simiae lack the enzyme required to further convert the niacin to niacin ribonucleotide. Reagent impregnated filter paper strips incubated in test medium produces yellow color which is indicative of niacin accumulation.

iv. **Nitrate reduction test**

MTB produces nitroreductase which catalyzes the reduction of nitrate to nitrite. Production of red color after adding sulfanilic acid and N-naphthyl ethylenediamine to an extract of the unknown culture is indicative of the presence of nitrite and a positive test.

v. **Tween 80 Hydrolysis**

This test is useful in identifying mycobacteria which possess lipase that splits Tween 80 into oleic acid and sorbitol. M. kansasii and M. gordonae hydrolyze Tween 80.

vi. **Catalase test**

The enzyme catalase splits $H_2O_2$ to release $O_2$ which is indicated by the presence of effervescence. The catalase activity after heating the culture at 68 °C for 20 min (heat stable catalase) is not seen in most strains of MTB. Semi-quantitative assessment of catalase activity is by measuring the height achieved by the column of bubbles produced when $H_2O_2$ is

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

added to tube culture. A column higher than 45mm is considered a positive test.

vii. **Arylsulfatase activity**

The enzyme arylsulfatase in mycobacteria breaks tripotassium phenolphthalein sulphate to release phenolphthalein. This free phenolphthalein turns the media pink in the presence of sodium bicarbonate. This test differentiates rapidly growing mycobacteria (positive) from group III non-photochromogenic mycobacteria (negative).

viii. **Urease activity**

The presence of urease activity is used to differentiate M. scrofulaceum (positive) from M. gordonae (negative).

ix. **Pyrazinamidase**

The enzyme pyrazinamidase deaminates pyrazinamide to form pyrazinoic acid which produce a red band in the culture media. This test is useful in distinguishing MTB, M. bovis, M. kansasii and M. marinum from other species of mycobacteria.

x. **Iron uptake**

M. fortuitum and M. smegmatis take up soluble iron salts from culture media to produce rusty brown appearance on addition of 20% ferric ammonium citrate. Other mycobacteria species lack this property.

xi. **Growth on MacConckey**

MacConckey supports the growth of rapidly growing mycobacteria. However, most other mycobacterium species cannot grow on this media.

xii. **Growth in 5% sodium chloride**

M. triviale and some strains of M. falvescens, M. fortuitum and M. abscessus can grow on egg based culture media containing 5% NaCl when incubated at 28 °C.

### 9.4. *Molecular methods*

There is a movement in clinical laboratories away from the conventional time consuming and tedious test for species identification of Mycobacteria recovered in culture e.g. nucleic acid probes have been produced to identify MTB, *Mycobacterium avium* intracellulare, M. kansasii and M. gordonae. There are 4 major applications used in clinical laboratories:

1. Use of DNA probes for culture confirmation of isolates recovered from clinical specimens.
2. Use of DNA sequencing for identification of mycobacteria.
3. Use of nucleic acid amplification tests (NAAT) for direct detection of MTB from clinical specimens.
4. DNA finger printing and strain typing of mycobacterium species.

1. **Nucleic acid probes**

This is a first nucleic acid based technology used to identify mycobacteria in positive cultures with very high accuracy, sensitivity and specificity. In this technique ribosomal RNA (rRNA) present in the cells and in culture in high quantities acts as genetic target. The radiolabeled (acridine ester) single stranded DNA probes hybridizes with rRNA forming stable DNA-RNA complex. After the inactivation of unhybridized probe, light generated is recorded by an instrument which is proportional to the amount of probe present. A predetermined threshold is used to determine positivity. This technique requires two hours.

2. **In situ hybridization**

This technology uses an oligonucleotide probe labeled with fluorescein and the interpretation is made by direct observation using fluorescence microscopy. It is popularly known as fluorescence *in situ* hybridization (FISH). If the detection of hybridized group is done by secondary reaction and color, the reaction is known as chromogenic *in situ* hybridization (CISH). FISH has been used to detect MTB in cultures and in direct respiratory samples that contain AFB.

3. **Nucleic acid amplification (NAA) methods**

In late 1990's FDA approved amplicor *M. tuberculosis* PCR assay (Roche diagnostics) and amplified *M. tuberculosis* direct test (AMTD) for respiratory specimens. These assays perform well on smear positive specimens but sub optimally on smear negative respiratory specimens, when compared with culture. Lately many in-house PCR and more recently real-time PCR assays have been developed and tested.

A study by Laraque F et al. tested performance of NAA on respiratory samples (N = 4642) and found that NAA had a sensitivity of 96% and specificity of 95.3% in specimen tested positive for AFB on smear.[53] In another study by Guerra RL et al. the effect of NAA results in clinical care of Pulmonary TB was evaluated. A total of 638 cases were included of which 270 were positive for MTB on culture. NAA had a sensitivity of 92.3% and specificity of 99.8%. NAA had decreased length of unnecessary therapy from 31 days to 6 days.[54]

**Polymerase Chain Reaction:**

PCR technique is now widely used in the research and diagnostic fields. This technique is based on amplification of specific DNA sequence to a large number of copies that can be detected by separation on gel electrophoresis. The amplification is achieved by using synthetic oligonucleotide primers complementary to specific DNA sequence. This process leads to a million fold amplification of target DNA through multiple cycles of:

i. Denaturation
ii. Annealing
iii. Extension

This results in an exponential increase in the number of copies of the target. A number of target genes of mycobacterial DNA have been evaluated for diagnosis by PCR and various other genotypic methods. The different DNA amplification targets used are – IS6110, devR, rpoB, IS986 and genes encoding MPB-64

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

(mpb64), 38kDa (pstS1), 65kDa (hsp65), 30kDa (fbpB), ESAT-6 (esat6), and CFP-10 (cfp 10) proteins. Any stretch of nucleic acid can be amplified by using DNA polymerase, provided that the specific sequence data are known to allow the designing of appropriate primers. The target most frequently amplified is the IS 6110 repetitive element which is present in multiple copies (up to 20) in most strains of *M. tuberculosis*. Species specific and genus specific PCR methods are being used with various targets and modifications of PCR.[55] Different studies by Abe et al.,[56] Clarridge et al.,[57] Beige et al.,[58] Nol te et al.[59] and Cheng et al.[60] have shown sensitivity of PCR to be 84%,86%,98%,91% and 72% respectively.

**Advantages of PCR:**

1. Most sensitive and rapid method of detection, can even detect when the bacilli number is as less than 10 {1—10 AFB/ml}
2. Determine rapidly whether AFB identified by microscopic examination in clinical specimens are *M. tuberculosis* or atypical mycobacteria.
3. Identify the presence of genetic modifications known to be associated with drug resistance.

**Disadvantages of PCR:**

1. False positive reactions-due to carry over contamination
2. False negative reactions-due to presence of inhibitors that interfere with the PCR
3. High cost
4. Amplification of DNA from both live and dead bacilli. So it cannot be used for monitoring therapy response.
5. Inhibition of amplification and reproducibility of the assay

The effectiveness of PCR for tuberculosis depends on experience and accuracy of the personnel conducting the assay. In many studies sensitivity of PCR has been compared with microscopy and culture results.

The disadvantages of PCR were addressed in a multi-laboratory study conducted by Noordhoek and coauthors. They had sent 200 sputum, saliva and water samples containing known numbers of M bovis BCG along with negative controls to 7 laboratories. Each laboratory used IS6110 insertion sequence as the target and their own protocol for PCR. High levels of false positive results ranging from 3% to 20% were reported. This was due to lack of monitoring of each step of the procedure. To overcome these problems there is a necessity of careful quality control during every step of assay.

**Real time PCR** is a technique that reduces the detection time and also quantifies the amount of M TB present in the clinical sample. The whole process of amplification and detection takes place in single reaction vessel in a closed system. Thus it reduces risk of amplicon contamination of laboratory. Since this technique is completely automated there is no need of post amplification processing and electrophoresis for detection of amplicons.[37]

4. **Line probe assay**

It is a reverse-hybridization technology made available commercially by Innogenetics and Roche diagnostics. It uses nitrocellulose strip on which multiple probes are immobilized. The amplicons are applied to the strip. Line or dots are formed at the site of amplicon-probe hybridization. The pattern formed at the end of reaction is then compared with standard key to interpret the results of that particular reaction. This technology is reverse of Southern blot technique with advantage of numerous probes can be tested simultaneously. Radioisotopes are not used which is another advantage of this technology over Southern blot. These assays are relatively simple when compare to DNA sequencing with simpler post-analytic analysis. It is used for species identification as well as detection of mutations that lead to drug resistance.[37]

5. **Transcription mediated amplification (TMA):**

TMA amplifies rRNA via DNA intermediate, producing billions of copies of RNA within an hour followed by detection using acridinium ester labeled DNA probes. Results are read in a luminometer in terms of Relative Light Units (RLU). Samples with values of 30 000 RLU are considered positive. TMA is sensitive enough to detect as little as 2.5 femptogram(fg) of RNA in clinical samples. Since there are 3—5fg of rRNA per *M. tuberculosis* cell, this assay is capable of detecting the rRNA contained in a single cell and is useful in conditions where culture is not feasible due to considerable time requirement or in paucibacillary.[61]

6. **DNA sequencing:**

This technology is very useful in the identification of slow growing organisms like mycobacteria. It is a complicated technique than simple probe hybridization and requires experience in sequence alignment, editing software and genetic data basis. In this technique, hypervariable A region of the 16S gene complex is most commonly targeted for rapid and accurate identification of mycobacteria. MicroSeq (Applied Biosystems, Inc, foster city, CA) has made this technology commercially available. The rpoB gene was popularly used as sequencing target because it provides identification information as well as information about the susceptibility to Rifampin.[37]

7. **Spoligotyping:**

This technique is used for studying genetic diversity and epidemiologic study of M TB strain circulating in a particular region. In this technology, DNA polymorphism is detected at direct repeat locus (DR locus) of M TB genome by hybridization assay which is then used for phylogenetic analysis. The predominant spoligopatterns reported from Indian studies are CAS, EAI, Beijing, manu. The technique further characterizes MTB strain and its importance in determining clinical manifestation.[62]

8. **DNA microarray analysis:**

High density oligonucleotide arrays (DNA microarrays) offer the possibility of rapid examination of large amounts of DNA sequences with a single hybridization step. This approach has recently been applied to simultaneous species identification and detection of mutations that confer drug resistance in mycobacterium. The DNA microarray is very promising method for the future because of following

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

i. Easy to perform
ii. Can be readily automated and
iii. It allows for identification of a large number of mycobacterial species in one reaction. This technique is expensive and currently used only for research work.[37]

9. **CBNAAT**(Cartridge Based Nucleic Acid Amplification Test)

**GENEXpert MTB/RIF assay:**

WHO has recommended GENEXpert MTB/RIF assay for the early diagnosis of TB and for the detection of resistant to Rifampicin in 2011. This simple cartridge based nucleic acid amplification test has revolutionized TB control program. It requires only 130 TB bacilli per ml of sputum for a positive result. It is also used for pleural, peritoneal, cerebrospinal, pericardial, and synovial fluid samples thus improving the diagnosis of extrapulmonary tuberculosis and detection of drug resistance.[63,64] This assay works on heminested PCR principle in a closed, completely automated cartridge based system which targets 81 bp fragment of rpoB gene for identification of M TB strain and subsequent probing of this region for mutations that detect Rifampicin resistance.[65] An overall sensitivity of GENEXpert MTB/RIF assay in the diagnosis of pulmonary TB was 88%, pooled sensitivity of 98% for smear and culture positive cases; 68% for smear negative cases.[66]

10. **PBMC circRNA detection**

In a study by Quin Z et al., peripheral blood mononuclear cell (PBMC) circular RNA (upregulated disproportionately) was used as diagnostic liquid biomarker to diagnose active pulmonary TB. In the study, it was found that PBMC circRNA levels were significantly higher within 5 pathways namely "cytokine–cytokine receptor interaction", "chemokine signaling pathway", "Fc gamma R-mediated phogocytosis", "neurotrophin signaling pathway" and "bacterial invasion of epithelial cells". The levels were increased in both young and old TB patients. A circRNA signature was developed based on above mention dysregulated pathways and was further validated by qRT-PCR, confirmed by microarray in 10 TB patients and 11 healthy controls. This technique has potential to be used as new tool in the diagnosis of active pulmonary TB.[67]

Extensive and detailed information regarding available as well as outdated diagnostic tests has been explained so far. However, all should have focus upon early diagnosis and prompt treatment of every cases of tuberculosis by following guidelines made available by Central Tuberculosis Division, Ministry of Health & Family Welfare.[68] The algorithm for diagnosis of pulmonary and extra-pulmonary tuberculosis cases as per Technical and Operational Guidelines for control of TB in India is shown in Figs. 3 and 4.

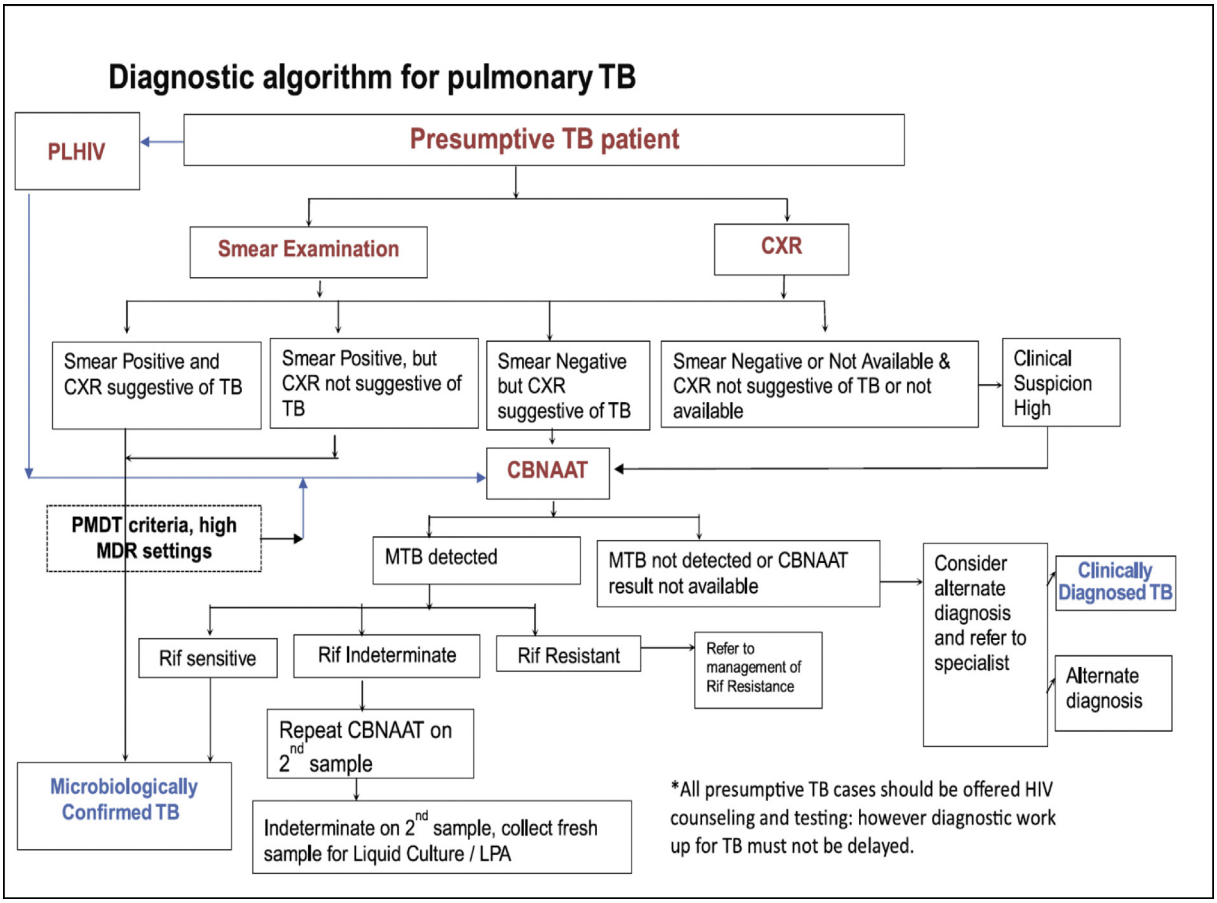



**Fig. 3 – Diagnostic algorithm for pulmonary TB.[68] PLHIV- People Living with HIV, CXR- Chest X-Ray, CBNAAT-Cartridge Based Nucleic Acid Amplification Test, LPA- Line Probe Assay.**

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

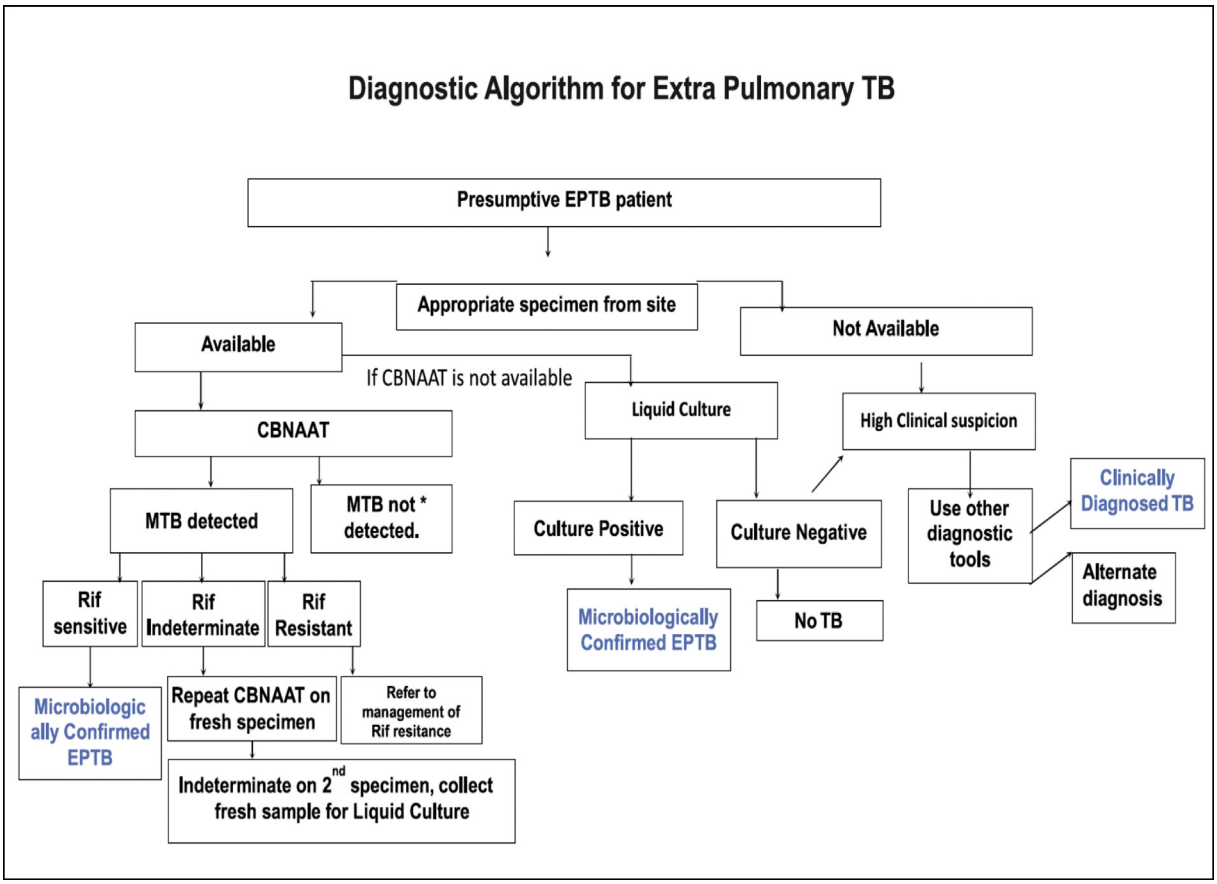



**Fig. 4 – Diagnostic algorithm for Extra Pulmonary TB.**[68]

## 10. Treatment[68]

RNTCP (now known as National tuberculosis elimination programme) has introduced daily regimen for drug sensitive TB in PLHIV, Pediatric TB cases in the entire country and for all TB cases in 104 districts. In drug sensitive TB, for all new TB cases, 8 weeks of intensive phase (IP) with Isoniazid(H), Rifampicin(R), Pyrazinamide(Z), Ethambutol(E) in daily doses as per 4 weight band categories whereas except Pyrazinamide, other 3 drugs are continued in continuation phase (CP) for another 16 weeks as daily doses.

For previously treated cases of TB, 12 weeks of IP with Isoniazid(H), Rifampicin(R), Pyrazinamide(Z), Ethambutol(E) and injection Streptomycin only for first 8 weeks of IP whereas 20 weeks of CP with Isoniazid, Rifampicin and Ethambutol (Table 3).

**MDR/RR-TB cases (with or without additional resistance):**

**Table 3 – Drug regimen for drug sensitive TB (prefix to the drugs stands for number of months).**

| Type of TB cases | Intensive Phase(IP) | Continuation Phase(CP) |
|---|---|---|
| New | (2) HRZE | (4)HRE |
| Previously treated | (2)HRZES+(1)HRZE | (5)HRE |

The CP may be extended by 12–24 weeks in treating EPTB (i.e. CNS TB, skeletal TB, disseminated TB etc) based on clinical assessment of treating physician.

The duration of treatment consists of 6–9 months of IP with Kanamycin(Km), Levofloxacin(Lfx), Ethionamide(Eto), Cycloserine(Cs), Pyrazinamide(z), Ethambutol(E), Isoniazid(H) and 18 months of CP with Levofloxacin, Ethionamide, Cycloserine, Ethambutol, Isoniazid on daily bases under supervision (Table 4).

### 10.1. XDR TB

All XDR TB cases are treated with injection Capreomycin(Cm), Moxifloxacin(Mfx), PAS, High dose Isoniazide(H), Clofazimine(Cfz), Linezolid(Lzd), Co-Amoxyclav for 6–12 months in intensive phase whereas except injectables remaining medications are continued for 18 months in continuation phase (Table 5).

Since the introduction of PMDT (Programmatic Management of Drug-resistant TB) in 2007, an increasing trend in diagnosis and treatment of MDR/RR TB cases was noticed till 2016 as shown in Fig. 5. In order to decentralize DR TB services (for easy accessibility, minimize patient travel and maximum patient satisfaction), a total of 628 CBNAAT centers were made operational by 2016 (Fig. 5).[69]

As per National Strategic Plan (NSP) 2017–25, following have been outlined[69]

1. Decentralization of diagnosis and treatment of MDR TB to district level
2. The recently endorsed WHO recommended second line probe assay

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

**Table 4 – Drug regimen for drug resistant TB (prefix to the drugs stands for number of months) modification of treatment*- All MDR-TB isolates should be treated with modification in treatment following liquid culture drug sensitivity testing for Kanamycin and Levofloxacin.**

| Type of TB cases | Intensive Phase(IP) | Continuation Phase(CP) |
|---|---|---|
| Rifampicin resistant + Isoniazide sensitive or unknown MDR TB | (6–9)Km Lfx Eto Cs Z E H<br>(6–9)Km Lfx Eto Cs Z E (modification of treatment*) | (18)Lfx EtoCs E H<br>(18) Lfx Eto Cs E |

**Table 5 – Drug regimen for XDR TB (prefix to the drugs stands for number of months).**

| Type of TB cases | Intensive Phase(IP) | Continuation Phase(CP) |
|---|---|---|
| XDR | (6–12) Cm Mfx PAS High dose I Cfz Lzd Amx/clv | (18) Mfx PAS High dose I Cfz Lzd Amx/clv |

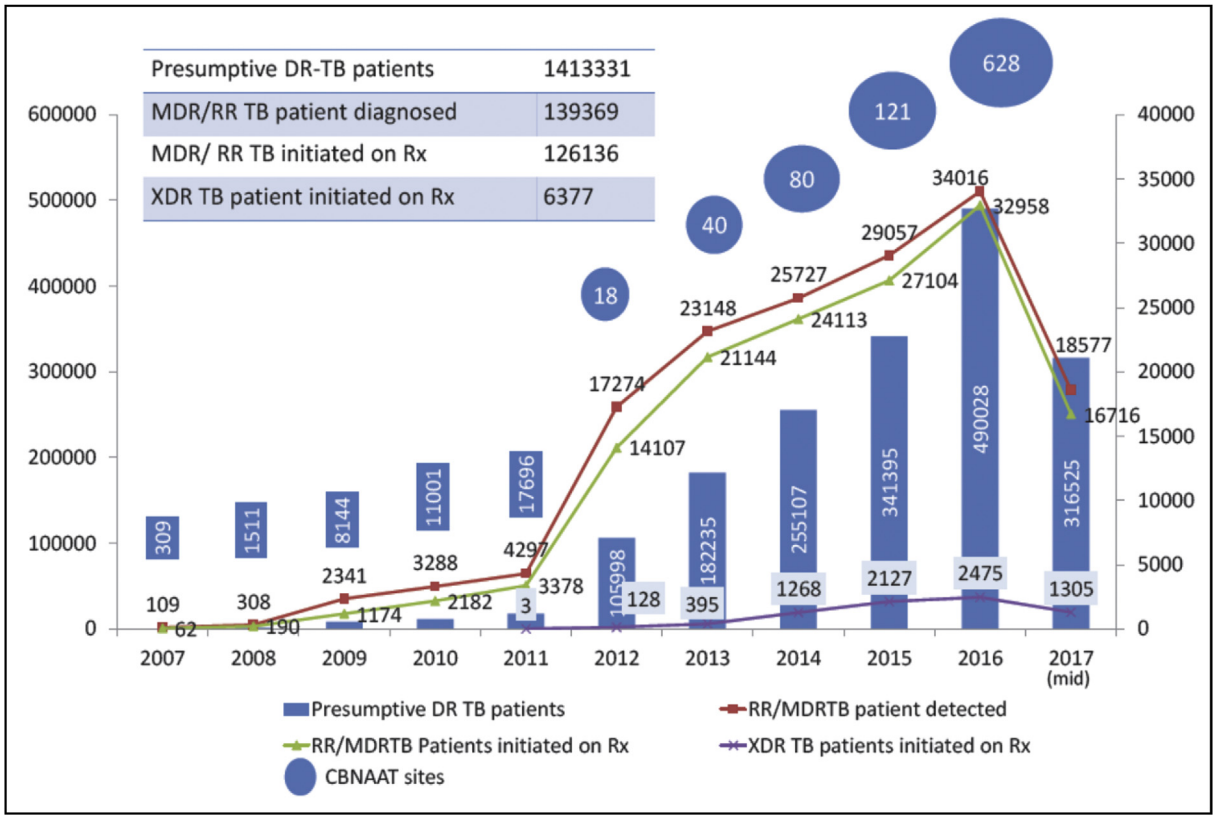



**Fig. 5 – Drug resistant TB cases and treatment initiation from 2007 to 2017.**[69]

3. Rapid molecular drug susceptibility test (DST) for second line drugs
4. Shorter MDR TB regimen
5. DST guided regimen to cover all variety of DR TB including Isoniazide mono-poly DR TB
6. Use of newer drugs like Bedaquiline
7. Revised recording reporting system i.e. e-NIKSHAY & pharmacovigilance systems for active drug safety monitoring & management

### 10.2. *Newer drugs*

Bedaquilline (Bdq), diarylquinoline class of drug which targets ATP synthase thus blocking supply of energy to MTb was added to MDR TB regimen from 2015 by WHO.

**Recommended dose:**

Week 0–2: Bdq 400 mg (4 tablets of 100 mg) daily (7 days per week) + optimized background regimen (OBR);
Week 3–24: Bdq 200 mg (2 tablets of 100 mg) 3 times per week (with at least 48 hours between doses) for a total dose of 600 mg per week + OBR; and
Week 25 (start of month 7) to end of treatment: Continue other second-line anti-TB drugs only as per RNTCP recommendations.[69,70]

Delamanid (Dlm), nitrpimidazole class of drug which blocks mycolic acid synthesis and releases nitric oxide upon metabolism poisoning MTb, has been introduced for the treatment of DR-TB in India (January 2018) after a series of

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

meetings and deliberations with national experts from government of India (GoI), WHO country office for India (WHO India) and key technical and developmental partners.

**Dosage:**

Week 0–24: Delamanid 100 mg (two tablets of 50 mg) orally twice a day + OBR
Week 25 to end of treatment: Continue other second-line anti-TB drugs (only as per RNTCP recommendations).[70]

## 11. Conclusion

It's been more than 100 years since the discovery of tubercle bacilli and the words of Robert Koch are still true,

> "amidst the persistently great variety in the ways and means of combating tuberculosis, it is yet necessary to ask what measures do indeed best satisfy the scientific requirements"[71]

Tuberculosis continues to challenge physicians, pathologists and microbiologists in every possible way and dilemma persists till today in early diagnosis and treatment of every form of it. WHO END TB strategy wishes to achieve 95% reduction in absolute number of tuberculosis deaths by 2035 which needs thorough understanding of tuberculosis and systemic filling of gaps in TB detection and treatment. The war is set on a platform of real knowledge; mankind equipped with experience of past and armed with present medicine to win against this ancient foe in its all forms. This review articles is a sincere effort towards increasing awareness about TB. I conclude by repeating the words of Sigmund Freud,

> "*One day, in retrospect, the years of struggle will strike you as the most beautiful.*"

## Authors contribution

Dr Arvind Natarajan: Analysis and interpretation of data, Revision of article for intellectual content.

Dr Beena PM: Analysis and interpretation of data, Revision of article for intellectual content, Final approval.

Dr Anushka Devnikar: Revision of article for intellectual content, Editing of article.

Dr Sagar Mali: Concept of study, Literature search, Acquisition of information and data, Drafting the article.

## Conflicts of interest

The authors have none to declare.

## REFERENCES

1. Ryan KJ, Ray CG. *Sherris Medical Microbiology*. 5th ed. USA: McGraw Hill company, Inc; 2010:489–505.
2. Sharma SK, Mohan A. Tuberculosis: from an incurable scourge to a curable disease- journey over a Millennium. *Indian J Med Res*. 2013;137:455–493.
3. Pommerville JC. *Alcamo's Fundamentals of Microbiology*. 7th ed. Canada: Jones and Bartlett; 2004:284–289.
4. Lee JY. Diagnosis and treatment of extrapulmonary tuberculosis. *Tuberc Respir Dis*. 2015;78:47–55.
5. World Health Organization. *Definitions and Reporting Frame Work for Tuberculosis: 2013 Revision*. Geneva: World Health Organization; 2013 (updated December 2014).
6. World Health Organization. *Global Tuberculosis Report*; 2018. Available from: http://www.who.int/tb/publications/global_report/en/.
7. Purohit M, Mustafa T. Laboratory diagnosis of extrapulmonary tuberculosis (EPTB) in resource- constrained setting: state of the art, challenges and the need. *J Clin Diagn Res*. 2015;9:EE01–EE06.
8. Lange C, Mori T. Advances in the diagnosis of tuberculosis. *Journal of Asian Pacific Society of Respiriology*. 2010;15:220–240.
9. Sharma SK, Mohan A. Extrapulmonary Tuberculosis. *Indian J Med Res*. 2004;120:316–353.
10. World Health Organization. *Commercial Serodiagnostic Tests for Diagnosis of Tuberculosis, 2011*. Geneva: WHO Press; 2011.
11. Sekar B, Selvaraj L, Alexis A, Ravi S, Arunagiri K, Rathinavel L. The utility of IS sequence based polymerase chain reaction in comparison to conventional methods in the diagnosis of extra-pulmonary tuberculosis. *Indian J Med Microbiol*. 2008;26:352–355.
12. Pingle P, Apte P, Trivedi R. Evaluation of microscopy, culture and PCR methods in the laboratory diagnosis of genito-urinary tuberculosis. *Am J Infect Dis Microbiol*. 2014;2:17–21.
13. Sudhindra KS, Prakash RC, Fathima F, Venkatesh VN, Rao A, Vijaynath V. Evaluation of PCR in early diagnosis of tuberculosis. *J Med Res Pract*. 2012;1:24–28.
14. Arvind N, Chand P, Baliga S, Bharati B, Shenoi S, Saralaya V. A comprehensive comparison of PCR based assay versus microscopy and culture in the diagnosis of tuberculosis in different clinical specimens. *Int J Biol Med Res*. 2014;5:3763–3768.
15. Cambau E, Drancourt M. Steps towards the discovery of Mycobacterium tuberculosis by Robert Koch, 1882. *Clin Microbiol Infect*. 2014;20:196–201.
16. Wallgren A. The time-table of tuberculosis. *Tubercle*. 1948;29:245–251.
17. Tille PM. *Bailey & Scott's Diagnostic Microbiology*. 13th ed. Mosby Inc; 2014:484–512.
18. Pfyffer GE, Palicova F. Mycobacterium: general characteristics, laboratory detection, and procedures. In: Versalovic J, Carroll KC, Funke G, Jorgensec JH, Landry ML, Warnock DW, eds. *Mannual of Clinical Microbiology*. 10th ed. ASN press; 2011:472–502.
19. Bhardwaj AK, Kumar D, Raina SK, Sharma S, Chander V. Assessment of extra pulmonary tuberculosis (EPTB) cases from selected tuberculosis units (TUs) of Himachal Pradesh, India. *Int J Health*. 2015;3:29–33.
20. World Health Organization. (2015). *Global tuberculosis report*. 20th ed. World Health Organization; 2015. https://apps.who.int/iris/handle/10665/191102.
21. Shirvastva AK, Brahmachari S, Pathak P, Kumar R, Patel U, Mandil A. Clinico- Epidemiological profile of extrapulmonary tuberculosis in central India. *Int J Med Res Rev*. 2015;3:223–230.
22. Raviglione MC, O'Brien RJ. Tuberculosis. In: Lango DL, Kasper DL, Jameson JL, Fauci AS, Hauser SL, Loscalzo J, eds. *Harisson's Principles of Internal Medicine*. 18th ed. USA: McGraw Hill company, Inc; 2012:1342–1344.
23. Fitzgerald D, Haas DW. Mycobacterium tuberculosis. In: Mandell GL, Bennet JE, Dolin R, eds. *Principles and Practice of*

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

*Infectious Diseases*. 6th ed. USA: Elsevier Churchill Livingstone; 2005:2852–2886.
24. Mohapatra PR, Janmeja AK. Tuberculous lymphadenitis. *JAPI*. 2009;57:585–590.
25. Vorster MJ, Allwood BW, Diacon AH, Koegelenberg CF. Tuberculous pleural effusions: advances and controversies. *J Thorac Dis*. 2015;7(6):981–991. https://doi.org/10.3978/j.issn.2072-1439.2015.02.18.
26. Ferrer J. Pleural tuberculosis. *Eur Respir J*. 1997;10:942–947.
27. Abraham P, Mistry FP. Tuberculosis of the gastrointestinal tract. *Ind J tub*. 1992:7992, 251.
28. Rathi P, Gambhire P. Abdominal tuberculosis. *J Assoc Phys India*. 2016;64:38–47.
29. Be NA, Kim KS, Bishai WR, Jain SK. Pathogenesis of central nervous system tuberculosis. *Curr Mol Med*. 2009;9:94–99.
30. Pigrau-Sarrallah C, Rodiguez-Pardo D. Bone and joint tuberculosis. *Eur Spine J*. 2013;22:556–566.
31. Hemal AK. Genitourinary tuberculosis. In: Sharma SK, Mohan A, eds. *Tuberculosis*. New Delhi: Jaypee brothers medical publishers; 2001:325–337.
32. Sharma SK, Mohan A, Sharma A. Miliary Tuberculosis: a new look at an old foe. *J Clin Tuberc Other Mycobact Dis*. 2016;3:13–27.
33. McAdam AJ, Milner DA, Sharpe AH. *Infectious Diseases in Robbins and Cotran Pathologic Basis of Disease. South Asia Edition*. 1. Reed Elsevier India pvt ltd; 2014:374–376.
34. Nicode LP. Immunology of tuberculosis. *Swiss Med Wkly*. 2007;137:357–362.
35. Wani RLS. Clinical manifestations of Pulmonary and extra-pulmonary tuberculosis. *SSMJ*. 2013;6:52–56.
36. Labh K, Sun X. Various manifestation of central nervous system tuberculosis:A review. *Biomed Lett*. 2016;2:1–7.
37. Winn W, Allen S, Janda W, et al. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. 6th ed. 2006:1064–1124.
38. Kapil A. *Ananthanarayan & Paniker's Textbook of Microbiology*. 9th ed. Chennai: Universities Press (India) Pvt ltd; 2013:352.
39. Shirvastava SR, Shirvastava PS, Ramasamy J. Revised national tuberculosis control program: progress in the diagnostic front. *J Mahatma Gandhi Inst Med Sci*. 2014;19:164–165.
40. Liu KT, Su WJ, Perng RP. Clinical utility of polymerase chain reaction for diagnosis of smear-negative pleural tuberculosis. *J Chin Med Assoc*. 2007;70:148–151.
41. Haldar S, Bose M, Chakrabarti P, et al. Improved laboratory diagnosis of tuberculosis — the Indian experience. *Tuberculosis*. 2011;91:414–426.
42. Derese Y, Hailu E, Assefa T, et al. Comparison of PCR with standard culture of fine needle aspiration samples in the diagnosis of tuberculosis lymphadenitis. *J Infect Dev Ctries*. 2012;6:53–57.
43. Padmavathy L, Rao L, Veliath A. Utility of polymerase chain reaction as a diagnostic tool in cutaneous tuberculosis. *Indian J Dermatol Venereol Leprol*. 2003;69:214–216.
44. Takahashi T, Tamura M, Asami Y. Novel wide-range quantitative nested realtime PCR assay for Mycobacterium tuberculosis DNA: clinical application for diagnosis of tuberculous meningitis. *J Clin Microbiol*. 2008;46:1698–1707.
45. Abbara A, Davidson RN. Etiology and management of genitourinary tuberculosis. *Nat Rev Urol*. 2011;8:678–688.
46. Mehta PK, Kalra M, Khuller GK, Behera D, Verma I. Development of an ultrasensitive polymerase chain reaction-amplified immunoassay (Immuno-PCR) based on mycobacterial RD antigens: implications for the serodiagnosis of tuberculosis. *Diagn Microbiol Infect Dis*. 2012;72:166–174.
47. Samant H, Desai D, Abraham P, et al. Acid-fast bacilli culture positivity and drug resistance in abdominal tuberculosis in Mumbai, India. *Indian J Gastroenterol*. 2014;33:414–419.
48. Carricajo A, Fonsale N, Vautrin AC, Aubert G. Evaluation of BacT/Alert 3D liquid culture system for recovery of mycobacteria from clinical specimens using sodium dodecyl (Lauryl) sulfate-NaOH decontamination. *J Clin Microbiol*. 2001;39:3799–3800.
49. Piersimoni C, Scarparo C, Callegaro A, et al. Comparison of MB/BacT ALERT 3D system with radiometric BACTEC system and Löwenstein-Jensen medium for recovery and identification of mycobacteria from clinical specimens: a multicenter study. *J Clin Microbiol*. 2001;39:651–657.
50. Angeby KA, Werngren J, Toro JC, et al. Evaluation of the BacT/ALERT 3D system for recovery and drug susceptibility testing of Mycobacterium tuberculosis. *Clin Microbiol Infect*. 2003;9:1148–1152.
51. Moore DAJ, Evans CAW, Gilman RH, et al. Microscopic Observation drug susceptibly assay for the diagnosis of TB. *N Engl J Med*. 2006;355:1539-50.
52. Yip CW, Leung KL, Wong D, et al. Denaturing HPLC for high-throughput screening of rifampicin-resistant Mycobacterium tuberculosis isolates. *Int J Tubercul Lung Dis*. 2006;10:625–630.
53. Laraque F, Griggs A, Slopen M, Munsiff SS. Performance of Nucleic acid performance test for Diagnosis of tuberculosis in a large Urban setting. *Clin Infect Dis*. 2009;49:46-54.
54. Guerra RL, Hooper NM, Baker JF, et al. *Chest*. 2007;132:946-951.
55. Raj A, Singh N, Gupta KB, Chaudhary D, Yadav A, Chaudhary A. Comparative evaluation of several gene targets for designing a multiplex-PCR for an early diagnosis of extrapulmonary tuberculosis. *Yonsei Med J*. 2016;57:88–96.
56. Abe C, Hirano K, Wada M, et al. Detection of Mycobacterium tuberculosis in clinical specimens by polymerase chain reaction and gene-probe amplified Mycobacterium tuberculosis direct test. *J Clin Microbiol*. 1993;31:3270–3274.
57. Clarridge JE, Shawar RM, Shinnick TM, Plikaytis BB. Large –scale use of polymerase chain reaction for detection of Mycobacterium tuberculosis in a routine mycobacteriology laboratory. *J Clin Microbiol*. 1993;31:2049–2056.
58. Beige J, Lokies J, Schaberg T, et al. Clinical evaluation of a Mycobacterium tuberculosis PCR assay. *J Clin Microbiol*. 1995;33:90–95.
59. Nolte FS, Metchock B, McGowan JE, et al. Direct detection of Mycobacterium tuberculosis in sputum by polymerase chain reaction and DNA hybridization. *J Clin Microbiol*. 1993;31:1777–1782.
60. Cheng VCC, Yam WC, Hung IFN, et al. Clinical evaluation of the polymerase chain reaction for the rapid diagnosis of tuberculosis. *J Clin Pathol*. 2004;57:281–285.
61. Shenai S, Rodrigues C, Mehta AP. Newer rapid diagnostic methods for tuberculosis: a preliminary experience. *Indian J Tubercul*. 2004;51:219–230.
62. Kandhakumari G, Stephen S, Sivakumar S, Narayanan S. Spoligotype patterns of Mycobacterium tuberculosis isolated from extra pulmonary tuberculosis patients in Puducherry, India. *Indian J Med Microbiol*. 2015;33:267–270.
63. Mbaave TP, Igbudu TJ, Egwuda L, Audu O, Jombo GT. GeneXpert MTB/RIF sputum MTB detection and human immunodeficiency virus seroprevalence among patients with presumptive pulmonary tuberculosis at a new teaching hospital in North-Central Nigeria. *Int J Curr Microbiol App Sci*. 2016;5:564–570.
64. Takhar RP. NAAT: a new ray of hope in the early diagnosis of EPTB. *Emerg Med (Los Angel)*. 2016;6:328. https://doi.org/10.4172/2165-7548.1000328.
65. Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of Rifampin resistance in pulmonary and extrapulmonary specimens. *J Clin Microbiol*. 2011;11:4138–4141.

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005

66. Pai M. Extrapulmonary tuberculosis: new diagnostics and new policies. *Indian J Chest Dis Allied Sci.* 2014;56:71–73.
67. Qian Z, Liu H, Li M, et al. Potential diagnostic power of blood circular RNA expression in active pulmonary tuberculosis. *EBio Med.* 2018;27:18–26.
68. *Technical and operational guidelines for TB control in India*; 2016. Available at: https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4573&lid=3177.
69. *Guidelines for PMDT in India*; 2017. Available at: https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4780&lid=3306.
70. *Guidelines for use of Delamanid in the treatment of drug-resistant TB in India*; 2018. https://tbcindia.gov.in/showfile.php?lid=3343.
71. *Nobelprize.org* Robert Koch—Nobel Lecture *Stockholm*. Sweden: Nobel Media; 2014. https://www.nobelprize.org/nobel_prizes/medicine/laureates/1905/koch-lecture.html.

Please cite this article as: Natarajan A et al., A systemic review on tuberculosis, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2020.02.005