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**REVIEW ARTICLE**

**RAPID DETECTION OF MDR- TUBERCULOSIS BY CBNAAT**

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**ABSTRACT**

Tuberculosis (TB) is one of the deadliest infections responsible for millions of deaths annually over the world from primitive times till date. India is one of the highest tuberculosis (TB) burden nations in the world accounting for nearly 23% of the worldwide rate constituting 11.4 million TB cases. India stands at second position in harboring multi drug resistant (MDR) TB cases, i.e., around 99,000 cases. Standard sputum based strategies to detect pulmonary tuberculosis include sputum microscopy and culture. However, these traditional methods result in a delay in the detection of TB and in consequence, beginning of the treatment.

To resolve such delays, in 2010, WHO endorsed a new molecular technique CBNAAT (Xpert MTB/ RIF) which is a fully automated diagnostic test that simultaneously detects tuberculosis and rifampicin drug resistance associated with mutation of rpoB gene within few hours. In this review we present a general overview of TB including the pathogenesis, diagnosis as well as ability of CBNAAT to detect Pulmonary TB in humans.

**Keywords***-* Tuberculosis, CBNAAT, Molecular Techniques, Standard Methods

1. **INTRODUCTION**

Tuberculosis (TB) is the most prevalent old illness of mankind and has co-existed with humans through several thousands of years [1].It is a long-lasting mycobacterial infection, often found with a latent period following primary infection. In humans, the primary cause of Tuberculosis is *Mycobacterium tuberculosis*, but a few other species, such as*M. microti, M. bovis, M. africanum, M. canettii, M. pinnipedii* as well as *M. caprae* also serve as infectious agents of TB. All these species combine together to construct the Mycobacterium tuberculosis complex (MTBC) [2].

*Mycobacterium tuberculosis* is a highly pathogenic [bacterium which](https://en.wikipedia.org/wiki/Bacteria) belongs to the family [Mycobacteriaceae](https://en.wikipedia.org/wiki/Mycobacteriaceae). Mycobacterium is a small (0.5 μm - 3 μm), slow-growing, aerobic bacilli. A complex, lipid-rich cell envelope distinguishes them in their classification as acid-fast and resistivity for Gram stain. It was discovered by Robert Koch in 1882 and for this discovery, the Nobel prize in Physiology or Medicine was granted to him in 1905 [3].

Tuberculosis is an original cause of infection and death globally, with death rate of approximately 1.7 million humans in 2016, mostly in low and middle income countries. HIV/AIDS is the most significant factor of TB infection and deaths in parts of the world where both infections are predominant. The most important cause is false-negative results and default identification of TB suspects in developing countries, as most programmes of TB control use staining such as Ziehl-Neelsen (ZN), which has low affectability and visits are needed which leads to complex default. On the other hand, mycobacterial culture generally needs 2-6 weeks time to yield a final result and requires proper arrangement and technical knowledge [4].

To overcome these shortcomings, a new molecular technique, Cartridge-based nucleic acid amplification test (CBNAAT) has been introduced by WHO in 2010, which relies on Polymerase Chain Reaction (PCR) method for TB detection and resistance to rifampicin. Its role in identifying TB in people having HIV has not been studied widely in India.

Gene Xpert test is based on nested real-time PCR with two uses:

(1) The identification of Mycobacterium tuberculosis complex DNA is identified in sputum specimens which are acid-fast bacilli (AFB) smear, positive or negative.

(2) The detection of Rifampicin resistance related mutations of the rpoB gene in samples from patients suspected of MDR-TB.

**2. REVIEW OF LITERATURE**

*Mycobacterium tuberculosis*, causing the infection of tuberculosis, was found by Robert Koch in 1882. It is an ancient disease with vast global health challenge and high burden on worldwide epidemiology among other contagious diseases. TB has been ranked second as a main reason for death after human immunodeficiency virus (HIV) and it has been found that one-third of the total world population is infected. In spite of the fact that contamination does not lead to active disease, 5-10% of the infected individuals develop active disease each year [5].The rest of the 90% people stay asymptomatic and can have inactive disease, but reactivation may happen any time during their life [6].

* 1. **History**

In 1819, Theophile Laennac of France detected the presence of consolidation, pleurisy and pulmonary cavitation as pathogenic signs of pulmonary or extrapulmonary TB [7]. In 1843, the German doctor Philipp Friedrich Hermann Klencke succeeded tentatively in the generation of human and bovine shapes of TB by immunizing tubercle materials into the liver and lungs of rabbits [8]. In order to isolate the TB bacillus, Theodor Albrecht Edwin Klebs sowed the tubercle material on egg white which was stored in sterile flasks,in1867.In his tests, the culture was rapidly sloppy and it was simple to recognize portable bacilli, which causes the illness in guinea pigs after vaccination into the peritoneal depression [9]. Later on, the renowned scientist Robert Koch isolated the tubercle bacillus using the methylene blue staining. The recognition, isolation and culturing of the bacillus was done in animal serum. Finally, he reproduced the infection by vaccinating the research facility animals with the bacillus [10].  This result was displayed to the Society of Physiology in Berlin on 24 March 1882 by him, which gave rise to a quantum leap in the battle against TB disease [3].

* 1. **Transmission**

Airborne particles, also known as droplet nuclei, are transmitted by infected individuals to vulnerable people which results in Tuberculosis (TB). These are 1–5 microns in diameter. These communicable droplet nuclei are minor droplets containing tubercle bacteria which are released when persons having pulmonary tuberculosis cough, sneeze, laugh, shout etc. These droplet nuclei stay in the surroundings for a few hours. Transmission takes place when an individual breathes in droplet nuclei containing tuberculosis bacteria. These droplet nuclei voyage into the upper respiratory tract via mouth and nasal openings. Thereafter, they move to the bronchi and at last to the alveoli and the lungs [11].

**2.3. Pathogenesis**

*M. Tuberculosis* causes the airborne bacterial infection of TB, which infects other body organs and most commonly occurs in the lungs [12]. *M. Tuberculosis* is exposed to the air as droplet nuclei from sneezing, coughing or shouting of a person having pulmonary TB. Once the bacilli reach the alveoli, they are engulfed by alveolar macrophages resulting in the elimination of a greater number of tubercle bacilli [13]. The unaffected proportion increases in the macrophages and is released when the macrophages destruct. These tubercle bacilli move via the blood flow or lymphatic passages to different body tissues or organs, most commonly to the lungs [14].

There are five stages of pulmonary tuberculosis as mentioned below. [15-18]

Stage I: No significant growth of bacilli. The bacilli are usually eliminated by alveolar macro phages. If the bacilli are not destroyed, they reproduce and alveolar macro phages are generally destroyed due to their multiplication.

Stage II: Commonly known as symbiotic stage where the bacilli multiply logarithmically within non-activated macrophages of developing abrasion, called tubercle.These non-activated macrophages, further, enter the tubercle from the main blood stream and are referred to as monocytes [18].

Stage III: In this stage, necrosis occurs and the bacilli become stationary. The growth of *M. tuberculosis* is inhibited by immune reaction to tuberculin like antigens that are released from bacilli [16].

Stage IV: This is the stage where the cell mediated immunity plays a prominent role to determine whether the disease turns out to be clinically recognizable.

Stage V: This is called liquefaction stage, where the bacilli escape the host’s defence mechanisms.

**2.4 Diagnosis**

The characterization and identification of tuberculosis from clinical samples is mainly based on biochemical characterization, acid fast bacilli (AFB), histopathology and isolation of *M. tuberculosis*. Earlier, the diagnosis of tuberculosis always remained a challenging problem especially in case of pauci-bacillary and extra-pulmonary forms. The commonest strategy of TB diagnosis is smear microscopy which was developed around 100 years ago. Smear microscopy requires acid fast bacilli (AFB) and10,000 to 1,00,000 organisms/ml. It can be saprophytic or else pathogenic mycobacteria [20]. The bacilli are examined in sputum smear under a microscope. It is classified as an [acid-fast bacillus as *Mycobacterium tuberculosis* retains stains after being treated with acidic solution](https://en.wikipedia.org/wiki/Acid-fast_bacillus)[21]. The [Ziehl–Neelsen stain](https://en.wikipedia.org/wiki/Ziehl%E2%80%93Neelsen_stain) and the Kinyoun stain are the most commonly used acid-fast staining techniques. [Auramine-rhodamine staining](https://en.wikipedia.org/wiki/Auramine-rhodamine_stain) [22] and [fluorescence](https://en.wikipedia.org/wiki/Fluorescence_microscope) [microscopy](https://en.wikipedia.org/wiki/Fluorescence_microscope) are also used.

Smear microscopy plays an important role in early diagnosis of Mycobacterial infections as the method is highly specific, rapid and the cheapest method used for detection of AFB in sputum. Recent advancement in smear microscopy is the arrival of fluorescence microscopy [23]. 10% higher sensivity has been discovered through this method when compared to Ziehl Neelsen (ZN) microscopy methods [24]. Fluorescent acid fast bacilli (AFB) is seen at a lower magnification and it takes 25% less time to observe smears compared to ZN smears. The sensitivity of microscopy is influenced by the quality of collected specimen, the quantity of mycobacterium present in the specimen, the processing method, the staining technique, and the quality of the observation [25]. As of late presented, Xpert MTB/RIF test could serve as a novel, fast, mechanized, and cartridge-based NAA test that can identify TB beside rifampicin resistance straightforwardly from sputum inside 2 hours of collection [26]. The GeneXpert cartridges are pre-loaded with all of the essential reagents for test preparing, DNA extraction, enhancement, and laser discovery of the opened up rpo B quality target. A major advantage of the Xpert MTB/RIF test is that it can be precisely administrated with negligible hands-on specialized time. This test in terms of affectability and specificity is worthy of use for TB discovery [27].

In a recent study of execution of Xpert MTB/RIF, among the 561 culture-positive patients (561/1730), a single, direct Xpert MTB/RIF test recognized 98.2% (551 out of 561) of the sputum smear-positive TB cases, as well as (124 out of 171) amounting to 72.5% of those with sputum smear-negative TB. The test was particular in 604 of 609 patients (99.2%) not influenced by TB. In patients with culture-positive, sputum smear-negative TB, a second Xpert MTB/RIF test increased detection sensitivity by 12.6% and a third by 5.1%, to reach 90.2%. The Xpert MTB/RIF measure accurately identified 97.6% (200 out of 205) of patients as compared to phenotypic DST harboring rifampicin-resistant strains and 98.1% (504 out of 514) of those with rifampicin-susceptible strains [28].

The WHO issued starting proposals on Xpert MTB/RIF, particularly for people suspected of having MDR-TB [29]. Xpert MTB/RIF has higher affectability for TB location in smear-positive patients than in smear-negative patients; in any case, this test may be important as an add-on test taken after microscopy in patients already found to be smear-negative [30].

* 1. **Epidemiology of Tuberculosis**

Tuberculosis (TB) has been ranked as the second leading cause of death among infectious diseases globally, after HIV [26]. In 2013, approximately 9 million people developed incident TB, whereas 1.5 million people died due to TB, including 0.4 million deaths among HIV positive individuals [26]. Though most of the TB cases and deaths were among men, but the burden of tubercular infection was also observed to be higher in women and it is increasing. In 2013, an approximate number of 3.3 million cases and 510,000 TB deaths occurred among women. Along with this, an estimated number of 550,000 TB cases and 80,000 deaths were reported among children [26]. Since 1993, World Health Organization (WHO) has declared TB as a global public health emergency. TB mortality rates have fallen by 45% since 1990 and incidence rates are also falling in developed countries. The estimated number of TB cases in 2013 in Asia and Africa were 56% and 29%, respectively.

India has ranked first among six high burden countries with the largest number of incident cases and has accounted for an estimated 24% of all TB cases worldwide [26]. The annual risk of infection was observed to be 1.5%, while the TB mortality rate per 100,000 population was found to be 24 (15-35) persons. About 4.2% (3.3-5.2) of HIV prevalence in incident cases was recorded [31], [29]. The status of TB in tribal communities is found to be relatively serious as compared to non-tribal population. Various studies in Sahariya have reported variable overall prevalence of TB, e.g., the first report published by Chakma *et al.* (1996) estimated the prevalence as 1270/100,000, which was followed by revised survey by Sharma *et al.* [32] (as 29.75/100), Rao *et al.* [33] (as 1518/100,000) and a recent revised estimate by Rao as 3294/100,000. Bhat *et al.* [33] reported the prevalence for TB in various tribes of Madhya Pradesh to be 387/100,000 population. Variable rates of prevalence of pulmonary tuberculosis has been reported for other Indian tribes also, e.g., in Pahadis of Kashmir valley it is 260/100,000 population [34], in tribes of Wardha district, Maharshtra the rate is 133/100,000 in Jawadhu tribe of North Arcot district, Tamil Nadu, it is 840/100,000 [35], in tribes of Car Nicobar, 140/100,000 and in Baiga tribe of M. P. it is reported to be 146/100,000 of population.

1. **CONCLUSION**

Diagnosis of tuberculosis in people is a challenging task requiring progressive advances, and determination. It is often troublesome to confirm microbiologically in portion because of the paucibacillary nature of the disease. Clinical conclusion needs standardization, and conventional and atomic microbiologic strategies need affectability, especially in people. Most of the tests make strides in affectability, but these tests cannot recognize tuberculosis infection from idle contamination and need specificity. These apparatus have promotes and serve as a tool of our capacity to identify Mycobacterium tuberculosis as well as MDR-TB.

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