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Review Article

Microbiology Section

Role of Mycobacterial Culture and Drug Sensitivity Testing Laboratory under National Tuberculosis Elimination Program for the Abolition of Tuberculosis in India by 2025

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ABSTRACT

India has made a bold promise to eradicate Tuberculosis (TB) by 2025 five years ahead of the global target. Although, one-fourth of the global burden with highest new cases of TB is shown by the country. So yet, no comprehensive analysis has been published on India's National Tuberculosis Elimination Program (NTEP) (2017-2025). The current review details the advanced diagnostic methods like Fluorescence Microscopy (FM), culture, nucleic acid amplification test (Cartridge Based Nucleic Acid Amplification Test (CBNAAT) and True Nucleic Acid amplification Test (TrueNAT)) and Line Probe Assay (LPA) as well as the role and network of mycobacterial Culture and Drug Sensitivity Testing (CDST) laboratories in national scaling-up of evidence-based policies and facilities, which is a critical component in India's fight against TB. The material of this study was mostly obtained from policy and program making documents of World Health Organisation (WHO) and annual TB reports of India. India's TB annual report 2021 says that only half of the patients were successfully treated in the period of conventional longer care regimens. The interventions to achieve the factors related patient's care have been implemented through universal drug sensitivity testing through CDST laboratories, which has driven therapy with a shorter regimen, newer medications, and social protection. In one hand, the comprehensive monitoring scheme through CDST laboratories for TB including all possible drug-resistance cases and other hand, patient's systemic treatment through shorter, more reliable and safer first- or second-line drug regimens are all necessary milestones to achieve the goal of our government for abolition of TB in India by 2025.

Keywords: Cartridge based nucleic acid amplification test, Culture and drug sensitivity testing, Drug resistant tuberculosis, Line probe assay, True nucleic acid amplification test

INTRODUCTION

Tuberculosis is an infectious disease mainly caused by bacteria known as *Mycobacterium tuberculosis* Complex (MTBC) [1]. Inspite of hard efforts taken to control TB by government and social platforms, this disease continues to be one of the major public health problems worldwide, particularly in developing countries [2]. According to WHO, an estimated 10 million people fell ill with TB altogether over the world which incorporates 5.6 million men, 3.2 million women and 1.2 million children among these India shares up to 25% of total cases [3]. TB tends to spread rapidly due to their asymptomatic existence including lack of early and reliable diagnosis become responsible for higher rate of morbidity and mortality. National services may do a better job of integrating existing diagnostic tools, but new and better tools are needed to allow low-cost, rapid and reliable TB screening closer to the point of treatment, as well as to ensure that all people at risk of TB receive the care they need [4].

The MTBC might be resistance against first line antitubercular drugs either isoniazid and rifampicin or both recognised as Multidrug Resistance (MDR-TB), however; resistance with not only by rifampicin and isoniazid but also with any fluoroquinoline (FQ) including any second line injectable drugs are counted as Extensively Drug-resistance (XDR-TB) [5,6]. Drug-Resistant Tuberculosis (DR-TB) has posed a relentless threat to successful TB control. Its existence has been recognised since the first anti-TB medicines were developed for the treatment of TB [7]. The introduction of MDR-TB and more recently XDR-TB has highlighted the need of possible required advanced DST and new medicines or their alternatives for their elimination [8]. To prevent the spreading of DR-TB, early and appropriate diagnosis and complete treatment with less Turnaround Time (TAT) is required [9]. Early detection has increased the mapping of high-risk populations and carefully designed systemic surveillance for active disease among them, which can be helpful to minimise the MTBC infection [10].

India has already taken many crucial measures with impressive and visionary policies in recent years to place itself as a pioneer for a TB-free nation [11-12]. Firstly, Government of India launched National Tuberculosis Programme (1962) followed by pilot programme Revised National TB Control Programme (RNTCP) in 1993 and fully launched 1997, now known as NTEP in 2020 with the aim of making India a TB-free country up to 2025 [13-15]. It is a remarkable and optimistic goal; nevertheless, achieving this status would require implementation of massive and large-scale diagnostic and treatment policies. It functions as a flagship component of the National Health Mission (NHM) and provides technical as well as managerial leadership to anti-TB activities within the country [16]. However, to tackle the large or undiagnosed issues, especially MDR and XDR-TB cases, the country requires high quality medical laboratories that can facilitate not only the diagnosis but also the drug sensitivity testing by covering the maximum cases throughout the endemic area of TB. Therefore, frequent diagnosis and proper treatment of DR-TB remains a major priority of our public health programme. The aim of this review was to summarise the NTEP endorsed different diagnostic methods and their role and laboratory networks towards the abolition of TB from India.

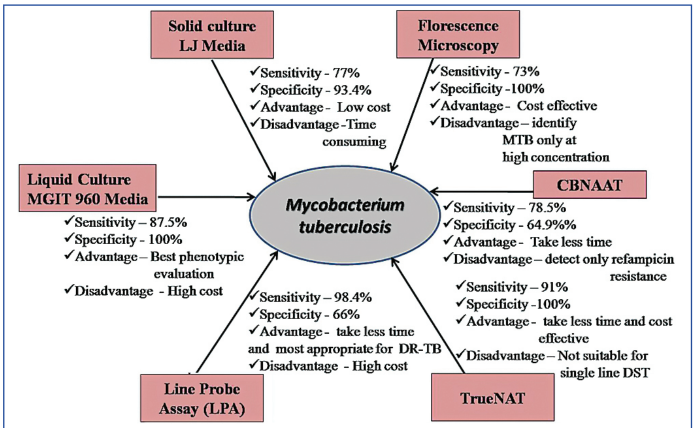
SELECTION OF REVIEWS

This review was based on information published on WHO annual TB reports as well as policy and programme documents of Central TB Division, Ministry of Health and Family Welfare, India [3,17].

For evaluating the efficiency and sensitivity of CDST laboratories for evaluation of *Mycobacterium* either it is genotypic or phenotypic, authors found out 35 article from PubMed and Google scholar as searched term "mycobacterial culture and drug sensitivity testing laboratory AND India". However, only 15 articles were present in internet and found suitable for CDST laboratories evaluation after thorough review. Authors have evaluated further 12 articles from google scholar for information about composition of media for solid and liquid culture and also adopted from guidelines given by WHO [18,19]. Definitions of different diagnostic methods were taken from textbooks of microbiology and information about genotypic technology TrueNAT was taken from Indian Council of Medical Research (ICMR) guidelines [20,21].

MAJOR DIAGNOSTIC METHODS UNDER NTEP

Molecular assays based on nucleic acid amplification techniques have been developed for the fast, sensitive, and reliable diagnosis of TB with the potential to determine their drug susceptibility status simultaneously [22]. Although, NHM provide the diagnostic services through a network of various types of laboratories in three tier fashion under the umbrella of NTEP [23]. In which, they constituted by facilities of microscopy, CDST like solid Lowenstein-Jensen (LJ media) and liquid culture (MGIT960), CBNAAT and TrueNAT and rapid molecular tests like LPA [10]. Their sensitivity, specificity, advantage and disadvantages and descriptions of these diagnostic methods are briefly discussed in [Table/Fig-1] [15,24].



[Table/Fig-1]: Mycobacterial culture and drug sensitivity testing (C&DST) laboratories based advanced techniques for detection of *M. tuberculosis* under National Tuberculosis Elimination Program (NTEP) [15,24].

(A) Fluorescence Microscopy (FM)

Microscopic analysis of clinical sputum specimens has been the major part of TB diagnosis over a century [25]. FM of sputum smear has been used to improve the sensitivity as compared to traditional Zeihl Neelsen (ZN) microscopy [26]. Direct microscopic analysis is frequently used method of TB diagnosis in low income countries like India. Sputum microscopy especially FM is not only affordable for diagnosis but also the determination of reaction to treatment of TB [27]. It retains the primary stain even after decolourisation as well as counter stain to highlight the MTBC for easier recognition [28]. In this technique, the use of Light Emitting Diode (LED)-FM is very helpful for the identification of smear-positive cases of MTBC among the heavy loaded Direct Microscopic Centres (DMCs) and medical colleges under the CDST laboratories [29]. The WHO reviewed the evidence for LED microscopy's effectiveness in 2009, using criteria suitable for assessing both the efficacy and the impact of new TB diagnostics on patients and public health [30]. LED-FM microscope is cost-effective, uses less energy and can be powered by batteries; additionally, the bulbs have a longer life span without harmful compounds if destroyed.

(B) Solid Culture Lowenstein-Jensen (LJ) Media

Solid culture media like LJ medium is a conventional method for CDST; it is less expensive and more readily available than other techniques. It is the most often used medium for culturing the MTBC recommended by the International Union against Tuberculosis [31]. It shows improved sensitivity over the smear with detection limit 100 bacilli/mL [32]. It is mainly composed of malachite green, glycerol and coagulated egg, in which, potassium dihydrogen phosphate anhydrous (KH_2PO_4), magnesium sulphate ($MgSO_4.7H_2O$) and magnesium citrate are also found in LJ medium to prevent gram positive and gram negative bacteria from growing and limiting growth to only *Mycobacterium* [18]. However, other bacteria are inhibited by the presence of malachite green in the medium. LJ culture showed weak growth rate of MTBC, at least 6-10 weeks for incubation including taken too much time for the result in comparison to liquid culture, is the limitation of this technique [20].

(C) Liquid Culture Media (BACTEC 960)

The BACTEC Mycobacterial Growth Indicator Tube (MGIT) 960 is a gold standard liquid culture method which is used as an in-vitro diagnostic instrument and resource-constrained environment as recommended by WHO [33-35]. It has been also approved for *M. tuberculosis* diagnosis in DST under the NTEP [24]. The MGIT 960 culture tubes contain 7 mL of Middlebrook 7H9 broth base, to which an enrichment supplement was added according to the instructions of the manufacturer, as well as mixture of antibiotics (MGIT PANTA) consisting of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin [36]. It is used especially for phenotypic diagnosis and drug susceptibility testing of TB, not only just for first-line drugs but also the second-line drugs among many laboratories of DST [19].

(D) Nucleic Acid Amplication Test (CBNAAT and TrueNat)

Nucleic Acid Amplication Test (NAAT) is offered the diagnosis of TB among children, high-risk population living with Human Immunodeficiency Virus (HIV) and extrapulmonary TB cases and also very useful among patients with TB who showed smear-negative through X-ray and preferable cases referred from private sector for early detection and appropriate treatment [37]. CBNAAT is an automatic cartridge-based molecular technique that detects MTBC as well as rifampicin resistance within two hours. It has been endorsed by WHO as an initial diagnostic test among patients suspected with MDR-TB in both pulmonary and extrapulmonary cases [38]. Unlike traditional NAATs techniques, CBNAAT or Xpert MTB/RIF cartridge show sample processing combines with Polymerase Chain Reaction (PCR) amplification and identification through single self-contained research device [38]. This assay covers the significant step forward platform and versatile tool for early diagnosis among all type of DR-TB cases in the fight against TB [39]. In other hand, chip based advanced technique like TrueNAT was developed by joint venture of Bigtec Laboratories and research and development subsidiary section of Molbio Diagnostics [40]. TrueNAT is cost effective when deployed at Point Of Care (POC) and also it is replacing smear microscopy as it can detect the cases more correctly [41]. This technique uses the real-time micro PCR technology and functional among wide range of environmental conditions with minimal user input in primary healthcare settings [21].

(E) Line Probe Assay (LPA)

The LPA is a reverse hybridisation procedure in which the patient's sample is hybridised with membrane strips coated with complementary markers of individual genes [42]. It is a group of Deoxyribonucleic Acid (DNA) strip-based tests that evaluate the MTBC strain's drug resistance profile. It work by examining the amplicons bind to wild-type DNA series probes that target the most common resistance related mutations to first- and second-line agents [31,43]. It also generates results very fast within 24-48 hours [44]. They can detect anti-tubucular drugs resistance status of both isoniazid and rifampicin through the identification of mutation in the *rpoB, katG*, and *inhA* genes [45]. In 2008, WHO approved the use of commercial LPAs for detecting MTBC in sputum smear positive specimens (direct testing) and cultured isolates of MTBC with drug resistance specimens (indirect testing) [44]. Using LPAs in countries with a high MDR/XDR strain allows for adequate, prompt care, lowering delivery speeds, morbidity and improving patient outcomes [46]. In recent advances among drug susceptibility testing, WHO also recommended this technique as initial test for second line-LPA for fluoroquinolones and injectable drugs resistance detection, instead of phenotypic culture [44]. Now, many NTEP laboratories use this technique for accurate and fast molecular DST assay for MDR and XDR-TB.

Role of Mycobacterial Culture and Drug Sensitivity Test (CDST) Laboratories

Laboratories are essential for monitoring the diagnosis and treatment of TB. Many laboratory techniques are used in detection especially microbial agent separation, causative bacteria, and drug susceptibility testing of isolates [47]. It becomes increasingly complex with the expansion of quality assured smear microscopy and novel CDST laboratory tools e.g., LPA and CBNAAT [43]. Since, multiple methods are needed to recover, classify and assess drug resistance for MTBC for the confirmation of any single case of TB [25]. Especially, treatment of MDR-TB is a difficult task that should be performed by qualified physicians in centres with reliable mycobacterial culture and in-vitro sensitivity testing services [48].

As per WHO hierarchical management system, NTEP play a significant role of *Mycobacterium* CDST laboratories as well as quality assurance organisation from the highest level of National Reference Laboratories (NRL) followed to State Intermediate Reference Laboratories, district/subdistrict level, and finally up to peripheral level of microscopy centres launched by Government of India [10]. Building on this vast laboratory network, Universal Drug Susceptibility Testing (UDST) was introduced freely or less expensive to patients for all type of drug resistance testing throughout the country. However, 100% CDST based quality assured laboratories with efficient capacity and timely identification of patients is the need of hour [49]. National Strategic Plan (NSP) (2017-2025) advocates the early identification of presumptive patients at the first POC among private or public sectors and highly sensitive diagnosis to provide the universal access of TB including DR-TB throughout the country [12,50]. If we see the India TB report 2021, conventional drug susceptibility testing of MTBC with liquid medium is well established and offers time saving and reliable results against a variety of first line and second line antituberculosis drugs [35]. Patients with high-risk of MDR-TB are diagnosed using WHO endorsed rapid diagnostics like CBNAAT/LPA/TrueNAT. However, response to treatment for MDR is always monitored by follow-up on liquid culture (MGIT960) system. Mostly laboratories including our institute Baba Raghav Das Medical College, Gorakhpur, India, also performed commercial Immunochromatic Test (ICT) for identification of *Mycobacterium* species in all detected cases [10].

All the patients on drug regimen also require TB culture because it helps to check whether the patient is taking his medicine in continuation or not. Since, no laboratory diagnostic is 100% full proof and also molecular detection depends on the presence of resistance conferring mutation. So, development of any new mutation cannot be detected by genotypic methods only and therefore, there is also need of phenotypic tests to identify drug resistance [23]. Modern techniques in laboratories for CDST like liquid culture or LPA etc can make our pathway easier towards the elimination of TB. It is a cost-effective and time-saving means of detecting MDR-TB, as well as a life-saving technique for early identification and treatment [6]. However, LPAs may not reduce the need for traditional CDST capabilities, as culture is still needed for conclusive TB diagnosis in smear negative patients, and DST is required for validation, if MDR/XDR-TB is not diagnosed [18].

Historically, MTBC was identified by phenotypic methods, such as morphological characteristics, growth rates, preferred growth temperature, pigmentation and series of biochemical tests. New phenotypic and genotypic susceptibility testing approaches includes the appealing of both first and second line drugs. Total 28,58,713 tests were performed by the CBNAAT and 1,25,923 tests by TrueNAT in which 53826 (7%) and 340 (3.1%) cases of DR-TB respectively among total confirm TB cases tested [Table/Fig-2]. The first line LPA detected 7.5% cases of MDR-TB and second line LPA detected 5.80% XDR-TB among total confirm TB cases tested. Total cases conducted by liquid culture are 2,85,775 and this second line DST detected approximately 5.2% cases of XDR-TB among 10184 cases [17]. The TAT has been further reduced by molecular detection of drug resistance and appears lower cost of testing followed to become the future of TB diagnosis in all the settings.

Genotypic diagnostic techniques	CBNAAT	Second line liquid culture	First line LPA	Second line LPA	TrueNAT
Total test conducted	28,58,713	11948	314570	58239	1,25,923
Number of detected cases (percentage) of *M. tuberculosis*	7,79,195 (27.26%)	-	289205 (91.9%)	50311 (86.4%)	11124 (8.83%)
DR-TB detection; number (percentage)	53826 (7.0%) R-resistant	620 (5.2%) XDR-TB	21739 (7.5%) MDR-TB	2920 (5.80%) XDR-TB	340 (3.1%) R-resistant

[Table/Fig-2]: Number of tuberculosis (TB) cases diagnosed by different C&DST based laboratories in 2020-21 [17].
C&DST: Mycobacterial culture and drug sensitivity testing; CBNAAT: Cartridge based nucleic acid amplification test; True NAT: True nucleic acid amplification test; LPA: Line probe assay; DR-TB: Drug resistance tuberculosis; MDR: Multidrug resistance; XDR: Extensively drug resistance; R-resistant: Rifampicin resistance

Network of Mycobacterium Culture and Drug Sensitivity Test (CDST) Laboratory

Laboratory networks with advanced diagnostic capability determine the efficacy of TB control programme in the new millennium including new technologies have made faster and more reliable of diagnosis, identification, and DST in developing countries like India [47]. The NTEP lab network's especially CDST laboratories are fitted with a variety of diagnostic technology for DR-TB diagnosis, including traditional solid culture and/or newer rapid TB diagnostic technologies, such as the LPA and liquid culture [51]. Existing detection methods include everything from basic smear microscopy and slow culture to advanced, expensive and technically complex molecular assays [23]. NSP (2017-2025) is based on huge network of all the three tier laboratories throughout the country for all the cases including all possible DR-TB [17].

The TB laboratory network has been expanded over the years to provide better access to quality assured diagnostic services [10]. If the analysis of number of laboratories under NTEP are done in these four to five years, we can see the efforts of government towards TB elimination [Table/Fig-3]. According to annual reports issued by India's Ministry of Health, there were 28 CDST laboratories in 2016-2017, but by 2020-21, the number had raised up to 87 laboratories. Same trends are also showing by the CBNAAT and TrueNAT laboratories [Table/Fig-3]. They are radio controlled by National Skilled Committee on identification and management of TB and the apex committee give the technical recommendation for the laboratory policy [12]. Further, NRL coordination committee reviews the progress and facilitates newer initiatives [52].

India has successfully created one of the largest TB laboratory networks in the world with 6 NRL, 31 Intermediate Reference Laboratories, 87 certified laboratories for Liquid Culture and Drug Susceptibility Testing services, and 64 certified laboratories for LPA services along with 21,717 Designated Microscopy Centres. These all laboratories support in the diagnosis of TB and provided patients

S. No.	Laboratories under NTEP	Total no. of laboratories established year-wise throughout the India				
		2016-17	2017-18	2018-19	2019-20	2020-21
1.	Florescence microscopy	13888	14000	16000	20356	21,717
2.	CDST	28	37	48	50	87
3.	NAAT (CBNAAT and TrueNAT)	628	628	1180	1530	3147
4.	LPA	54	56	62	64	64

[Table/Fig-3]: Network of different laboratories status as per annual TB reports of India (2017-2021) [10,17,49,51].
NTEP: National tuberculosis elimination programme; C&DST: Mycobacterial culture and drug sensitivity testing; CBNAAT: Cartridge based nucleic acid amplification test; True NAT: True nucleic acid amplification test; LPA: Line probe assay

with access to effective treatment depending on their drug resistance patterns. The Indian government has taken several measures to eradicate tuberculosis. At 8000 DMCs, the NTEP programme aims to substitute smear microscopy with upfront molecular testing using NAAT for TB diagnosis. There are currently 3000 NAAT platforms in the NTEP programme, as well as 18 CDST laboratories being built and 28 CDST laboratories being upgraded with LPA [17].

CONCLUSION(S)

To achieve the vision of a TB free India, the NSP proposes ambitious policies with ample funds to completely abolish the TB cases in India by 2025. Three years are only ahead for this nationwide end TB commitments set out in the sustainable development goals. But still there is need of scale-up free, highly sensitive diagnostic tests. Although, there is provision of universal tests for drug-resistant TB (UDR) by the help of CBNAAT and TrueNAT including especial cases of MDR and XDR-TB through LPA. But more numbers of laboratories are still needed to complete the goal of its elimination. Incidence and mortality rates caused by TB have recently declined, but many cases of DR-TB remain undiagnosed or ineffectively handled by unskilled healthcare service providers in India. To tackle this problem Government of India should declare TB as a public health emergency and launch a campaign to fight it. Aside from aggressive TB prevention campaign, stricter and faster diagnostic procedures, as well as continuous or periodic survey of drug resistance, will be preventative measures of chemotherapy. It will also serve as a helpful parameter among previous and current NSP programmes for achieving the abolition of TB in India by 2025.

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