REVIEW ARTICLE   **OPEN ACCESS**

Tuberculosis Diagnosis and Detection of Drug Resistance: A Comprehensive Updated Review

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Abstract

Tuberculosis (TB) is a significant public health challenge, especially in developing nations. Developing a TB eradication strategy is hampered by the global health concern of drug-resistant (DR) TB. Effective patient treatment, preventing TB transfer and avoiding the upsurge of DR strains depend primarily on the timely and accurate identification of DR TB. Due to inadequate sensitivity, the necessity of trained laboratory personnel, the sluggish growth pattern of *Mycobacterium* bacilli in culture, and the small number of bacilli that are usually found in extrapulmonary TB samples, TB diagnosis is still tricky in clinical practice. Although mycobacterial culture is the gold standard to identify TB and determine drug resistance, it takes 2 to 8 weeks to develop. Despite their high cost, nucleic acid amplification tests (NAATs) and whole-genome sequencing (WGS) are the commonly employed molecular-based methods for diagnosing and identifying TB. The WHO suggested the GeneXpert MTB/RIF to identify TB and detect resistance to rifampicin. In comparison, numerous molecular techniques were developed, including allele-specific PCR (MAS-PCR), solid-phase hybridization, real-time PCR (RT-PCR) and droplet digital PCR-based technique (DDPCR). This manuscript is intended to overview the current approaches for the phenotypic and genotypic diagnosis of TB disease and identifying resistance to antitubercular drugs depending on recently published articles, WHO and CDC reports, and commercially available diagnostic tools.

Keywords: *Mycobacterium tuberculosis*, TB, Drug Resistance, Molecular Techniques, MDR-TB

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INTRODUCTION

Tuberculosis (TB) disease, caused by the bacterium *Mycobacterium tuberculosis* (MTB), is one of the world's most fatal infectious illnesses. According to a recent estimation, MTB infects around one-fourth of the world's population and kills 1.5 million individuals annually.[1] TB was considered a primary reason for mortality from a single infectious agent until the pandemic coronavirus disease 2019 (COVID-19). Still, according to the World Health Organization (WHO) reports, TB is a worldwide prevalent health issue, with new cases rising from 7.1 million in 2019 to 10 million in 2020. MTB-affected people exhale germs into the air (e.g., by coughing and sneezing), which spreads the disease; therefore, air represents a primary source of illness in humans.[2] Thus, the delay in the treatment of the active cases of TB leads to the further spread of the bacterium to others. Accordingly, early and accurate TB diagnosis and drug susceptibility determination are crucial. In the following sections, TB disease, its risk factors, and the available methods for diagnosing and detecting drug resistance, including the traditional and recent techniques, will be comprehensively reviewed.

TB disease, management and health risk factors

TB is a contagious disease caused by the infection with MTB. TB illness usually affects the respiratory system, causing pulmonary tuberculosis (PTB), but it can also affect other body organs causing extrapulmonary tuberculosis (EPTB). Adults are the most affected by TB, around 90% of patients, with more cases among men than women.[2] The tubercle bacilli settle in the airways once the person inhales droplet nuclei formed from the evaporation of respiratory droplets. When the immune system cannot control the infection, it will spread locally within the lungs and nearby lymph nodes within three to eight weeks. Patients with PTB will likely experience dyspnea, cough, hemoptysis, irregular chest radiographs, night sweats, weight loss, anorexia and exhaustion. Over the past several years, more cases of EPTB have been reported. EPTB may affect several organs or parts of the body, including lymph nodes, pleura, central nervous system, eyes, musculoskeletal system and both genitourinary and gastrointestinal tracts. Therefore, the clinical manifestations of EPTB cases are determined according to the affected body site. EPTB patients may have stomach discomfort (most common), diarrhoea, infertility, monoarticular joint pain, headache, meningism and lymphadenopathy. However, EPTB patients are less likely to experience the typical clinical features of PTB.[3]

Macrophages are essential to the human host's innate immune response to MTB. Still, the ability of MTB to live over extended periods within macrophages in a tubercle granuloma is a crucial pathogenic trait. Macrophages can kill mycobacteria by various mechanisms, including apoptosis, immune-inflammatory reactions and phagocytic activity.[4] Conversely, the pathogen can evade and/or resist the host defences, ensuring survival and persistence by delaying phagosome-lysosome fusion, providing a favourable environment for tubercle bacilli survival and reproduction. Correspondingly, macrophages have developed other defence tactics against MTB, including activating autophagy to promote phagosome-lysosome fusion and bacilli clearance.[5]

The human immune responses to MTB infections may either disrupt bacterial development and eradicate the bacteria or, in most cases, cause latent tuberculosis infection (LTBI). Still, 5% to 15% of LTBI patients can develop active TB disease, including pulmonary or extrapulmonary illness. Active TB usually appears immediately after getting the organism, although it might occur years later in some instances due to debilitated immunity.[6] WHO published worldwide programmatic management recommendations for drug-resistant TB in 2014. The recommended treatments included rifampicin (RIF), ethambutol (EMB), pyrazinamide (PZA), and levofloxacin for six months in RIF-susceptible and isoniazid (INH)-resistant TB infections. However, injectable medications, such as streptomycin (STR), are not recommended in patients with this RIF-susceptible and INH-resistant TB.[7]

Various predisposing factors for PTB, particularly for infection with multidrug-resistant TB (MDR TB), have been identified. These risk factors include two past bouts of PTB and illness that lasted more than 60 days, sputum acid-fast bacilli (AFB) smear score of 3+, and chest radiographs showing cavities or pleural effusion.

However, international studies from different countries have identified variable records of these risk factors. Therefore, identifying risk variables and the possible impact of the geographic area are required to establish optimum measures for MDR TB control. In addition, to limit the MDR TB cases, physicians should ask for an early drug susceptibility test (DST) and give appropriate treatment in response to the clinical variables and radiographic chest abnormalities.[8]

Many reasons constrained the systematic reviews and meta-analyses of MDR TB risk factors. The reason for that is most investigations are a single-regional emphasis, making it impossible to evaluate the impact of the risk influences globally. In addition, the risk factors are studied from the perspective of either the host or the pathogen. Thus, a comprehensive evaluation to identify MDR TB risk factors should be carried out across all geographic regions to enhance international efforts to control MDR TB effectively.[9] Many studies from different countries explored MDR TB risk factors, including Indonesia, Vietnam, Russia, and China.[10-13] In China, MTB strains are likely to be MDR, linking the MTB Beijing genotype strain with past TB treatment or failure of remedy. In addition, MTB strains in Beijing showed more virulence in animal models, with more widespread tissue damage, faster expansion, and high mortality rates.[13] The proposed possibilities for this link are the differences in cell wall construction and greater virulence, resulting in inadequate intracellular drug concentrations and more prolonged, persistent infections.[10] Notably, higher incidence rates of TB have been documented in certain people groups than others. There are two categories concerning illness with MTB and getting TB disease: people at high risk for exposure to or getting infected with MTB and people at higher hazard for getting TB disease after infection with MTB (Table 1).[14]

Transmission and epidemiology of TB

TB commonly affects the lungs, and it is an air-borne disease. PTB patients exhale the MTB bacteria when they cough, sneeze or spit. To get infected, a person only has to breathe in some MTB

Table 1. High-risk groups for tuberculosis infection and disease[14]

People at high risk for infection with MTB [14]
- Contacts of persons who have been diagnosed with TB or suspected of having the disease
- People who were born in or regularly visited countries with high rates of TB, such as Mexico, Philippines, Vietnam, India, China, Haiti, and Guatemala, or any other nation with increased rates of TB
- People now reside in or have previously lived in high-risk communal settings (for example, homeless shelters or penal institutions)
- Employees that work in crowded high-risk environments, such as workers in the healthcare field who assist patients with TB
- People with immunocompromising diseases
- Locally defined communities with a higher prevalence of LTBI or TB, such as medically underserved, low-income people, and drug or alcohol abusers
- Infants, children and adolescents exposed to adult patients are at risk for LTBI or TB disease

People at increased risk for developing TB disease after infection with MTB [14]
- HIV-positive people
- Children under the age of 5 years old
- People who have recently been infected with MTB (within the past two years)
- People who have had untreated or improperly treated tuberculosis in the past
- Patients on immunosuppressive therapy, such as tumour necrosis factor-alpha (TNF-α) antagonists, systemic corticosteroids like $\geq$ 15 mg prednisone per day, or immunosuppressive medication therapy after organ donation
- Persons with silicosis, diabetes, chronic renal failure, leukaemia, or head, neck, or lung cancer
- Individuals who have undergone gastrectomy or a jejunoileal bypass
- Persons with low body mass index
- Those who smoke cigarettes and those who misuse drugs or alcohol
- Medically underserved, low-income populations and populations characterized locally as having an elevated prevalence of MTB illnesses.

bacteria. When a person has active TB disease, the symptoms might be minor for months. Therefore, there might be delays in obtaining treatment, leading to the further spread of the bacterium to others. Consequently, over one year, a person with active TB can infect 5 to 15 persons through intimate contact.[15]

TB is one of the most widespread illnesses worldwide. Effective management and control of TB disease necessitate a broad knowledge of epidemiology and transmission risk factors.[16] MTB is estimated to infect over two billion individuals (almost one-fourth of the world population). Nearly 10 million people have TB disease annually, with 1.6 million deaths. Accordingly, TB is the world's most significant cause of mortality due to bacterial infection.[14] Nearly 70% of TB cases worldwide exist in Southeast Asia and Africa. Even though the total number of cases in Southeast Asia was more prominent, the overall incidence in both areas was comparable (226 per 100,000 in Southeast Asia and 237 per 100,000 in Africa). In 2017, most high-incidence countries were in Southeast Asia and Africa; nevertheless, the percentage of TB cases among HIV-positive people in Africa (27%) was more significant than in Southeast Asia (3%). While the incidence of TB in Europe remains low, the proportion of TB patients with rifampicin-resistant TB (RR TB) or MDR TB

was much higher than in the other areas (range = 3.6% – 6.3%).[14]

Globally, TB frequency decreased by 8.5% from 2015 to 2019. The decrease was from 142 cases of a newly developed active TB illness for every 100,000 people in 2015 to 130 cases per 100,000 in 2019, or an annual reduction rate of roughly 2.1%. The WHO End TB Strategy set its target of a 20% cumulative decrease by 2020. However, TB is still a significant infectious disease that causes death globally, which is higher than the mortality rate of acquired immunodeficiency syndrome (AIDS) caused by HIV infection, compared to a 14% decrease in the death rate from 2015. It fell off the WHO target of reducing TB-related deaths by 35% by 2020, with an estimated 1.4 million TB-related deaths, while 1.2 million among those who were HIV-negative and about 200,000 were positive for HIV).[17]

Diagnosis of TB disease and identification of MTB

An early, quick, and precise diagnosis of TB, or identification of MTB and determining the antimicrobial susceptibility of infecting strain, are essential for better treatment and management. This section discusses the conventional and advanced methods for TB diagnosis or identification of MTB and DST.
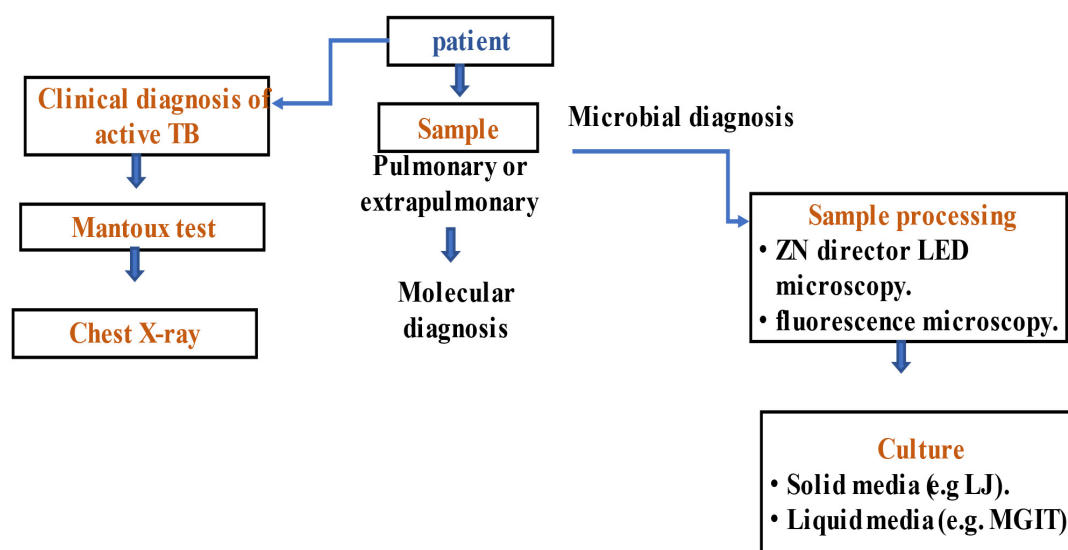
**Figure 1.** Overview of the current TB diagnostic scheme

Conventional methods for diagnosis of TB

Three main approaches are utilized to diagnose TB: sputum smear microscopy, MTB culture and chest radiography. The smear microscopy sensitivity varies, especially in individuals with other infections like HIV patients. Due to MTB sluggish growth, the traditional culture procedures for MTB isolation, identification and drug susceptibility testing take several weeks. However, prolonged, inappropriate antituberculosis therapy contributes to developing resistance in MTB to available antitubercular drugs, which reduces the number of available treatments and increases the length and expense of TB therapy and morbidity and mortality rates. Therefore, innovative diagnostic techniques are necessary to quickly identify MTB and determine the drug susceptibility profile of the infecting strain. Accordingly, several molecular-based methods have been developed, which are more precise and have shorter turnaround times than the phenotypic methods. However, even though these techniques yield data a few hours after sample collection, MTB culturing and phenotypic susceptibility testing are still required to quantify a particular strain's sensitivity to certain antibiotics and determine its drug susceptibility.[18] Acid-fast smear microscopy, nucleic acid amplification tests (NAATs), and culture-based procedures are currently used for the definitive laboratory diagnosis of TB (Figure 1).

Clinical diagnosis of active TB

The individual in contact with a PTB patient for at least two to three weeks is suspected of having the disease.[19] However, MTB infection is frequently asymptomatic in healthy persons with no comorbidities affecting their immunity. This TB infection is LTBI, which implies the patient has no active TB infection but might get it at some point (known as TB reactivation). It is assessed that one in every three persons worldwide has LTBI, and 5 to 10% of these people are at risk of TB reactivation. In addition, most people with LTBI will get TB within the first five years after getting infected with MTB. However, when predisposing conditions are present, the likelihood of reactivation rises significantly. When an infected person has any disease threatening his health, such as HIV/AIDS or malnutrition, LTBI escalates to active TB infection.[20] PTB can be diagnosed when a patient exhibits the following signs and symptoms: weakness, fever, evening or night sweats, a persistent cough with sputum (phlegm), and chest discomfort. Lung
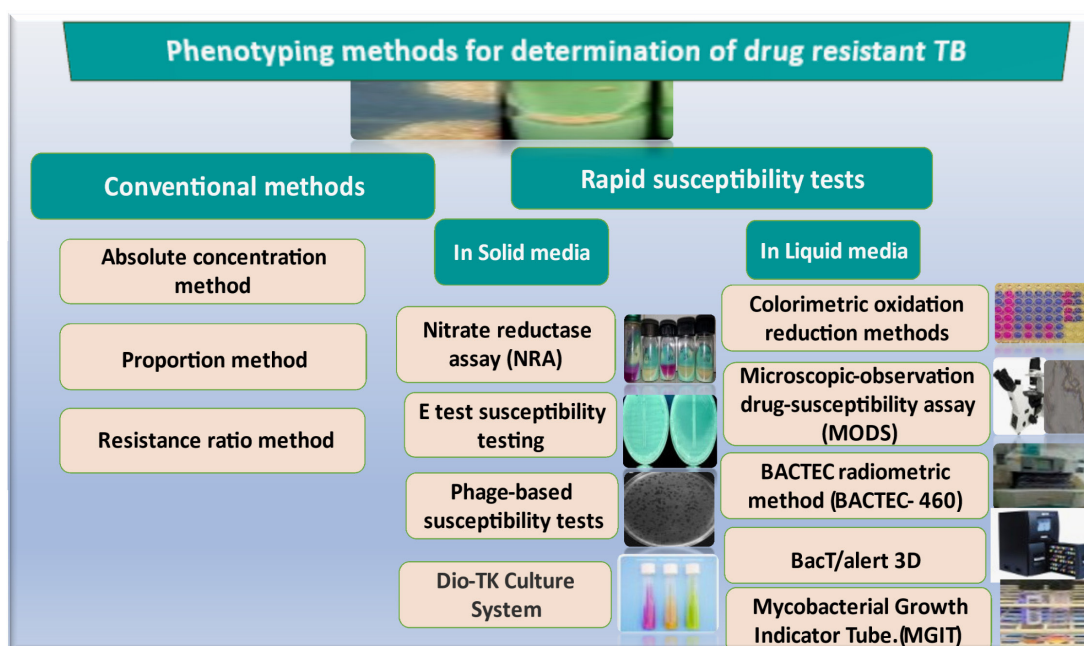

Figure 2. Phenotypic methods for determination of TB resistance

secretions are usually accompanied by blood or hemoptysis, the spitting of blood derived from the lungs or bronchial tubes hemorrhage, weight loss, pale complexion and bright sunken eyes in severe cases.[21] A positive Mantoux test confirms the PTB diagnosis. In addition, an advanced PTB is indicated by the appearance of lung lesions (caverns) in a chest X-ray.[22]

Mantoux test

The Mantoux test (or the tuberculin skin test) assesses a patient's tuberculin sensitivity.

In this test, a tiny amount of a mycobacteria protein extract or purified protein derivative dissolved in glycerol is injected intradermally (5 units of tuberculin in 1 mL) on the forearm. The halo of erythema (skin redness) should be considered when measuring the diameter of the skin induration (thickened, hard skin) between 48 and 72 hours after injection. It is a positive response when the induration diameter is 5 to 15 mm. The positive test indicates that the individual has been exposed to MTB infection, but it does not necessarily mean he is sick. Even though the
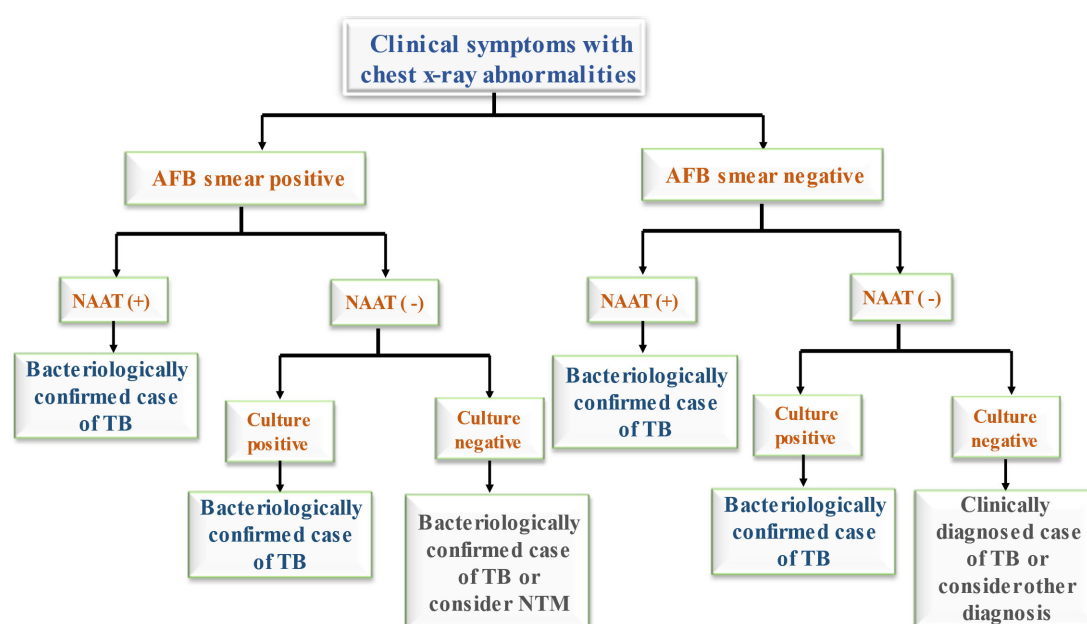
Figure 3. The role of nucleic acid amplification tests (NAATs) in the diagnosis algorithm of TB. TB: tuberculosis, NAAT: nucleic acid amplification test, NTM: nontuberculous mycobacterium.[109]
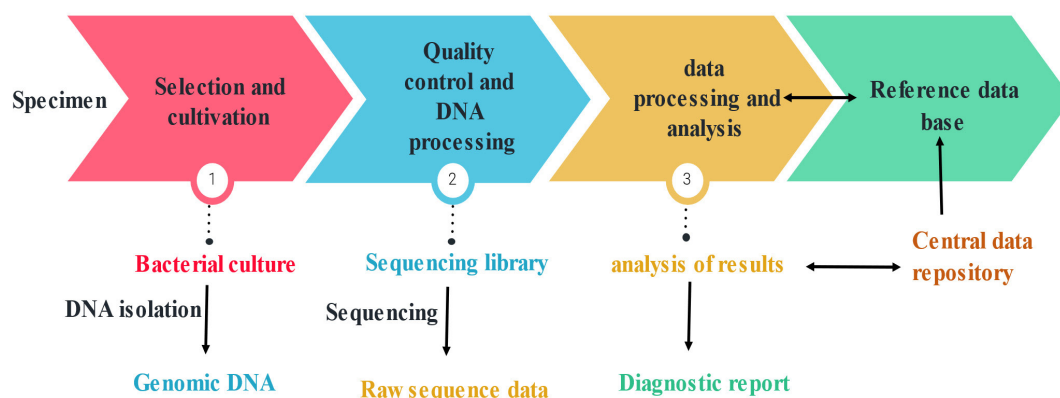
Figure 4. Whole-genome sequencing workflow of MTB from specimen processing until the diagnostic report

risk factors or medical history of TB, it is worth considering whether or not the person has the disease. Notably, persons allergic to tuberculin or vaccinated with the BCG TB vaccine may give false-positive results. In contrast, if a PTB person has comorbidities such as AIDS or has compromised immunity, false-negative results are probable.[23]

Chest X-ray

When a person shows a positive tuberculin test with no apparent symptoms, chest radiographs should be employed to exclude the potential of PTB. The chest X-ray is a critical diagnostic of TB or suspicion of TB patients. This X-ray shows the parenchymal infiltrates, hilar adenopathy, cavitation, nodules, and pleural effusion.[24] Also, any aberration in HIV/AIDS patients or other immunocompromised people might suggest tuberculosis. On the other hand, the chest X-ray may seem completely normal. In addition, old cured tuberculosis lesions frequently manifest as pulmonary nodules in the hilar region or higher lobes, with or without fibrotic scarring, volume loss, and bronchiectasis (enlargement of parts of the airways within the lung). Scarring of pleura might also be evident. Notably, slowly growing tubercle bacilli may be seen in nodules and fibrotic scars, potentially leading to active TB. Regardless of age, people with these lesions should be considered high-priority candidates for latent infection therapy if they have positive tuberculin skin test results. However, calcified nodular lesions (calcified granuloma) have a minimal probability of evolving into active TB.[25]

Artificial intelligence-supported interpretation for chest X-ray radiography

The lack of experienced radiologists and the high inter- and intra-radiology reader interpretation variability have hampered the performance of chest radiography, particularly in areas with an increased incidence of TB and limited access to high-quality medical care.[26,27] Lately, artificial intelligence (AI)-aided diagnostics systems have developed and evolved at an unprecedented rate, with many commercial systems available for clinical usage. These systems can potentially address existing limitations of chest X-ray radiography, including reducing human inter-reader variability and reproducibility and

providing radiologic services where radiologists are unavailable.[28] Many medical image-analyzing AI algorithms based on deep learning and deep convolutional neural networks were being used for radiograph reading at the same time.[29] AI evaluates radiographs and expresses anomaly scores in computer-aided detection products that suspect TB.[30] The diagnostic performance of these computer-aided detection software was comparable to that of a human reader interpreting digital chest radiography, with a sensitivity ranging from 90-92% and a specificity ranging from 23-79%.[28,30] WHO has just conditionally endorsed CAD as an alternative to human interpretation of digital chest radiography for TB screening and triage in 15-year-old adults and older.[30] The most studied software is CAD4TB (Delft Imaging Systems, Netherlands) version 6. When compared with PCR amplification tests, CAD4TB has been shown to have from 90 to 100% sensitivity and from 23 to 45% specificity in detecting TB.[31]

Traditional laboratory-based methods to identify PTB

Traditional laboratory techniques for identifying MTB are still widely utilized because they are both feasible and trustworthy. Identifying MBT using AFB staining and fluorescence microscopy and the cultivation and isolation of mycobacteria are diagnostic approaches that allow for a more precise diagnosis of PTB than the clinical-based diagnosis. Because many of the former clinical features of PTB might be mistaken for other illnesses, such as coccidioidomycosis, in addition to a PTB clinical picture, detecting and characterizing MTB in patient sputum is necessary.[32] In other words, for an accurate diagnosis, the clinical diagnosis of PTB is essential first, the confirmation or rejection by the laboratory-based methods.[22]

Identification of acid-fast bacilli (AFB)
Ziehl-Neelsen (ZN) staining technique

It is already long-established that the Gram staining approach does not work well with mycobacteria. Thus, Ziehl-Neelsen (ZN) staining was utilized for detecting AFB, which relies on direct observation of AFB by microscopy. Nonetheless, because certain bacterial species, such as *Nocardia* genus members, are also AFB, ZN is neither 100 % specific nor a sensitive

diagnostic method of MTB.[33] More than 120 AFB mycobacteria species are not PTB causal agents. Further, some mycobacteria other than MTB produce atypical pulmonary symptoms.[34] The ZN staining process includes the following steps. The culture in suspension, or a liquid biological sample, is placed on a slide and dried, then fixed using a heat air flux. After submerging the fixed bacteria-containing slide in a phenol-carbol fuchsin solution, the smear is heated to allow the dye to penetrate the waxy cell wall of mycobacteria and binds to mycolic acids. After staining, the dyed preparation is washed with water and decolourized byan acid-decolorizing solution (1% hydrochloric acid in isopropyl alcohol or methanol) to remove the red dye from any non-AFB cells. Because of the characteristic waxy lipid coating of AFBs, only AFBs, including mycobacteria, will retain the phenol-carbol fuchsin dye. Malachite green or methylene blue counterstaining is used to stain non-AFB material that failed to hold the initial dye. Afterwards, a microscope will be used to observe the difference between the red AFB and the non-AFB material, green or blue hue. This process may differ owing to the variation in structure across mycobacteria genera. *M. ulcerans*, for example, has a robust AFB phenotype, but *M. leprae* has a mild AFB phenotype. *M. ulcerans* may be decolourized with 3% ethanol, but *M. leprae* requires 0.5 – 1% sulfuric acid. The staining and discolouration timeframes vary from one *Mycobacterium* genus to the other. In addition, this technique requires skilled persons.[35]

Fluorescence microscopy (FM) technique

The fluorescence microscopy (FM) technique is an alternative to ZN staining, employed where the required facilities are available. FM is more rapid than ZN because it is easier to see MTB bacilli. FM is also, as a minimum, 10% more sensitive than traditional light microscopy.[36] That is important because the rapid turnaround time can be critical in identifying presumptive TB illness in high-volume laboratories. In addition, other test factors, such as the time length of staining and the fluorescence background, play a role in the effectiveness of laboratory reports.[37] FM technique is a semi-automated approach, while ZN must be performed and investigated manually, which requires a skilled

operator. However, the ZN method is available in low-resource laboratories than FM, particularly in developing countries, because fluorescence microscopes are significantly more expensive than light-field microscopes. Auramine and rhodamine are the fundamental stains used in FM to identify mycobacteria in biological materials. However, these dyes are non-specific fluorochromes that bind to the mycobacterial wall mycolic acids. The dyed mycolic acid resists discolouration by alcohol-acid solutions. The potassium permanganate as a counterstain avoids non-AFB fluorescence; thus, the test is specific and minimizes the artifacts. AFBs can be seen under an epifluorescence microscope once the staining is complete. When exposed to UV light, AFBs sparkle in yellow or brilliant orange on a dark background. An AFB smear is examined using a fluorescent microscope equipped with a 20 x or 40 x objective and a 100 x oil immersion objective lens to illustrate the morphology of fluorescing organisms.[38]

Currently, novel technologies for recognizing mycobacteria in clinical samples have been developed based on light-emitting diode (LED) technology. This LED technology is far less expensive than the original auramine and rhodamine systems.[39] The LED-based approach involves using a 'Royal Blue' LuxeonTMLED to illuminate a standard fluorescent microscope, showing that this type of lighting is adequate for detecting auramine O-stained *Mycobacterium* spp. The developers argue that their approach is low-cost, low-power, and safe and that LEDs' dependability makes them a viable replacement for mercury vapour lamps.[40] Conclusively, despite using the AFB stain without a mycobacteria culture, AFB visualization has a weak negative predictive value when utilizing ZN staining or FM. Thus, AFB culture should be combined with an AFB stain since the latter has a significantly larger negative predictive value.[38]

MTB culture

Mycobacterial culture is still the gold standard for diagnosing TB, owing to its high sensitivity and specificity, mainly when molecular techniques are unavailable. MTB culturing enables species identification, drug susceptibility testing, monitoring the response to therapy, andstudying disease epidemiology. However,

MTB cannot be grown on standard culture media and must be cultured on specialized media for a long incubation period. Thus, MTB culturing is a costly procedure.[41,42] In addition, the sluggish growth rate of MTB prevents early diagnosis and management of the disease. Three types of culture media can isolate and support the growth of MTB from biological samples, including the Lowenstein-Jensen (LJ) medium and its adaption for the Ogawa-Kudoh technique and Egg-based medium.[43] Agar-based media (Middlebrook 7H10 and 7H11) and liquid medium (Middlebrook 7H9 and MGITs) are also available. Although, the LJ medium is the commonly used solid medium for growing mycobacteria. The commercially available liquid-based culture systems may be manual, semi-automated, or automated using colourimetric or fluorometric detection techniques. Depending on the design and the source of the material, the Middlebrook 7H9 or the MGIT medium is frequently utilized. The Septi-Chek AFB systems (Becton Dickinson, Sparks, MD, USA), the ESP (Extra Sensing Power), Myco-ESP Culture System II (Trek Diagnostic Systems, USA), and the BacT/ALERT MB (bioMˈrieux, Marcy-l'Etoile, France) are an example of these systems. Other systems include the BACTEC MB9000, BACTEC MGIT 960, and 320, and the most widely used is the BACTEC MGIT 960 system.[18]

Biomarker-based serodiagnosis
Serum biomarker-based technologies

Biomarker-based serodiagnosis might be beneficial for identifying TB in those hard-to-diagnose groups like pediatric TB patients, extrapulmonary TB and HIV co-infected patients. The WHO approved C-reactive protein (CRP) as a screening marker in HIV-infected TB patients. CRP is sensitive but not specific; it may be helpful when combined with other biomarkers.[30] Several protein biomarkers have been discovered and could be efficient in TB diagnosis, such as interferon-γ, interferon-γ inducible protein-10, tumour necrosis factor-α, fibrinogen, α2-macroglobulin, matrix metalloproteinase-9, transthyretin and complement factor H. These combined protein biosignatures demonstrated 92% sensitivity and 72% specificity for detecting TB.[44] Antibodies, cytokines, and ribonucleic acid (RNA) signatures are the most commonly evaluated host biomarkers for TB diagnosis. Host biomarkers seem sensitive but often have less specificity than pathogen biomarkers.[45]

Antibodies were the most studied host immune biomarkers. More recent studies showed promising results for antibodies as signatures for TB diagnosis.[46] Antibody-based assays are preferred as they are low-cost and require limited operator training. TB-specific IgG4 has been shown to correlate with disease activity and decrease after treatment.[47] Several companies are developing innovative antibody-based technologies, including MBio Diagnostics Inc. (Boulder, USA) and TB Biosciences (Bethlehem, USA).[48]

Another approach lies in the determination of host T cells as biomarkers such as cluster of differentiation (CD) 27, CD38, or CD153, as well as human leukocyte antigen (HLA)-DR and cell proliferation markers such as Kiel (Ki) using flow cytometry.[49,50] These markers have repetitively shown sensitivity and specificity levels compatible with the targeted product profile (TPP) defined by the WHO, targets for a confirmatory TB test, particularly for children.[51]

Other biomarker-based technologies

Detection of TB biomarkers in urine is a noninvasive helpful approach to diagnose TB in patients unable to expectorate sputum as elderly, children and adults without a productive cough.[52] The Alere Determine TB LAM Ag fast test (Alere Inc., Waltham, USA) detects LAM and assists in identifying TB in highly immunocompromised people with HIV. The LAMP test is a straightforward strip test that takes only a few minutes to complete. On the other hand, LAM tests have limited sensitivity in individuals with CD4 counts larger than 200 cells/µl, and the WHO does not recommend their use.[53] The fact that the LAM test is not specific for MTBC presents a hurdle. Methods for detecting enzymes are also being developed. Global Bio Diagnostics Corp. in Texas, USA, has designed a biphotonic detection technology that uses a fluorescence reporter enzyme to identify live TB bacteria that produce β-lactamase.[54]

Early MTB infections can be detected using volatile organic compounds (VOCs) in the breath. Using VOCs may aid in diagnosing MTB in HIV patients and children. The possibility of employing VOCs for diagnosing TB was verified

in gigantic African pouched cane rats practised to recognize VOCs in TB-infected sputum. Compared to smear microscopy, VOCs performed better in this proof-of-concept investigation.[53] Commercial VOC-based solutions are being developed or evaluated, such as Aenose (eNose Company, Zutphen, The Netherlands), BreathLink (Menssana Research Inc, Newark, USA), and TB breathalyzer (Rapid Biosensor Systems, Cambridge, UK). However, a limited amount of data supports it.[54]

Phenotypic methods of determination of antimicrobial drug susceptibility of MTB

MTB has become resistant to the most clinically available antitubercular drugs. Drug-resistant MTB, particularly MDR MTB (showing resistance to as a minimum RIF and INH) and extensively drug-resistant MTB (XDR MTB), which is also resistant to fluoroquinolones and kanamycin or amikacin or capreomycin.[55] The incidence of drug-resistant TB is increasing worldwide, making MDR TB treatment difficult and costly, with many adverse effects on patients. Furthermore, drug resistance is a significant barrier to efficient prevention and control of TB.[56] Thus, TB is a global public health problem and one of the top ten mortality diseases worldwide.[57]

Previously, WHO recommended a universal policy for controlling TB called the directly observed treatment short-course (DOTS) approach.[58] The strategy is effective if the MTB strain is not drug-resistant, i.e., sensitive to the first-line anti-TB drugs (INH, RIF, EMB, and PZA). However, the challenge arose when both MDR and XDR expanded globally. Accordingly, the DOTS-plus strategy for patients with MDR TB was established. It involves using the second-line anti-TB drugs (fluoroquinolone and kanamycin/amikacin/capreomycin), although they have serious side effects.[59] Therefore, determining drug resistance of MTB to first- and second-line anti-TB drugs is crucial to both TB therapy and control.[60]

Knowing each clinical MTB isolate's entire drug susceptibility profile would allow for more individualized short-course effective therapy while minimizing exposure to unuseful and potentially harmful drug effects.[61] Thus, the regular performance of DST for MTB-infecting strains might help prevent and treat drug-resistant TB; even DST for at least RIF is recommended.

In addition, all concerned people must perform more actions and efforts to control MDR TB before it becomes a pandemic with terrible consequences.[62] However, a significant challenge is facing developing countries to train and hire experienced laboratory technicians and implement suitable diagnostic laboratories with modern devices and a continuous supply of expensive diagnostic kits.[38]

Determination of drug resistance in MTB by conventional phenotyping methods

Phenotypic methods are the gold standard for the DST of MTB. However, these methods are time-consuming and require specialized laboratories and well-trained personnel to prepare media with various antitubercular drug concentrations, specimen processing and culturing. Three traditional methods use solid media: proportion, absolute concentration (minimal inhibitory concentration (MIC)), and resistance ratio. Phenotypic methods are efficiently employed to determine the sensitivity of MTB to the first-line anti-TB drugs, including RIF, INH, STR, and Ethambutol (EMB) (Figure 2).[63,64]

MTB proportional DST method

The proportion method compares growing a regulated inoculum of mycobacteria on a drug-free medium to growing on culture media with an anti-TB drug's critical concentration.[64,65] The concept of the MTB proportional DST technique is as follows. Suppose 1% of the inoculum on the drug-containing medium comprises resistant mutants. In that case, these mutants will only grow, and by dividing the number of colony-forming units (CFU) by those appearing on the drug-free medium, the microorganism can be identified as sensitive (≥ 1%). The number of susceptible CFUs on the drug-containing medium should be less than CFUs on the drug-free medium. This method takes around 4 to 6 weeks to get the results. It can be applied directly on the sputum or indirectly using mycobacterial culture. The proportion method using Middlebrook 7H10 agar has been the "gold standard" method for several decades.[66]

Absolute concentration method

In this method, an inoculum of MTB is

cultured on LJ medium, or Middlebrook 7H10 agar has serial dilutions of anti-TB drug and is on drug-free media. Drug resistance is revealed by the lowest drug concentration inhibiting growth, i.e., minimal inhibitory concentration (MIC), which is indicated by obtaining less than 20 colonies by the end of incubation for four weeks.[67] In this test, a standardized inoculum is cultured on media containing gradient concentrations of tested anti-TB drugs. The resistance is expressed in terms of MIC. However, the test is significantly affected by inoculum size and the capability of the MTB microorganisms to grow.[64]

Resistance ratio (RR) method

The resistance ratio (RR), an old method, includes determining the MIC of the tested drug to the MTB isolate in relation to the MIC against the drug-susceptible reference microorganism, such as *M. tuberculosis* strain H37Rv; both are tested simultaneously.[68] Drug resistance is expressed as the resistance ratio of the MIC for the test MTB isolate divided by that for the standard strain. After incubation, growth is verified as the presence of 20 colonies or more, and MIC is the lowest drug concentration where less than 20 colonies are obtained. A ratio of 2 or less designates a sensitive strain, and a ratio of 8 or more indicates a resistant strain.[69]

Determination of drug resistance of MTB by rapid susceptibility test on solid media
Nitrate reductase assay (NRA)

In NRA, critical concentrations of the drug are tested. Changing the medium colour to pink and purple represents MTB ability to grow and, thus, resistance to the drug. The principle of this assay measures the reduction capability of nitrate to nitrite by MTB in LJ media with the same concentrations of anti-TB drugs used in the proportion method.[70] The appearance of a pink or purple colour upon adding Griess reagent to the culture medium indicates the resistance to the drug.[71] The NRA test is simple to set, reads the results and gives precise information on drug susceptibility. In addition, technicians get the results quicker than waiting for the visual detection of colonies.[72]

E-test drug susceptibility testing

E-test comprises strips containing concentration gradients of anti-TB drugs (AB BIODISK, Solna, Sweden). The antimicrobial agent disseminates from the strip into the medium, thus inhibiting the growth of susceptible strains. The MIC is determined, and the isolate is interpreted as resistant or susceptible.[73] However, this method may give false results compared to conventional LJ proportion methods. That is because the diffused drugs degrade in the sluggishly growing mycobacteria, resulting in an unclear MIC cut-off point. Additionally, it needs a heavy inoculum (equivalent to McFarland 3 turbidity standards).[65,74]

Phage-based susceptibility testing

Phage-based susceptibility testing relies on the ability of live drug-resistant MTB to allow the growth of an infecting mycobacteriophage. The mycobacteriophage, a virus that infects mycobacteria, is pre-incubated with the test anti-TB drug before adding MTB, which forms plaques on the agar surface when MTB is resistant.[75] Mycobacteriophage-based susceptibility assays are divided into two types; the first is the phage amplified biologically (PhaB) assay, which employs sensitive host bacteria such as *M. smegmatis* to multiply offspring phages capable of infecting *M. tuberculosis*.[76] FASTPlaqueTBTM, a commercially available kit, offers greater sensitivity and specificity than standard MTB diagnostic methods and can more precisely represent MTB activity, metabolic features, and the proportion of drug-resistant bacteria.[77] The second type is Bronx Box, which is based on fluorescent reporter phage and can decrease the time of DST testing to three days for INH, RIF, capreomycin and EMB. Mycobacteriophage-based assays have several advantages, including low cost, reduced detection time, ease of use, infecting only living bacteria and decreased false positive results. Conversely, it might be technically challenging, labour-intensive and have significant failure rates with uninterpretable results.[65,75,76]

Dio-TK culture system

Dio-TK Culture System, a rapidly automated colourimetric technique, is commercially available to isolate mycobacteria from clinical samples.[78]

This system contains dye as an indicator, and colour variations in the media reveal the metabolic activity due to mycobacterial growth. The culture media with different antitubercular drugs start red, change to yellow if MTB resists the specific drugs, and turn green if the sample is contaminated. Compared to solid media like LJ-medium, colour shifts appear sooner, thus reducing the time it takes to produce the results by around half (from six weeks to about three weeks).[79]

Determination of drug resistance of MTB by rapid tests in liquid media
Colourimetric oxidation-reduction methods

The colourimetric assays for detecting drug resistance of MTB are tests that employ oxidation-reduction reactions to produce a colour shift. These tests use the liquid medium 7H9 broth in 96-well microtiter plates.[80] In addition, these tests use resazurin, an oxidation-reduction indicator dye, and tetrazolium bromide or 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT), a redox indicator reduced by dehydrogenases produced when cells are still alive.[81] In these tests, MTB isolates are inoculated into 7H9 broth enriched with the test anti-TB drug and incubated at 37°C for seven days. Then, Alamar blue or resazurin reagent is added to wells. If MTB grows in the presence of the drug, it will reduce the blue reagent to a pink dye that may be seen with the naked eye or measured with a colourimeter. The blue-to-pink colour change in a drug-containing well suggests the existence of resistant MTB.[82] The colourimetric methods have been evaluated in many studies with reported sensitivity and specificity ranges of 94 - 100%, with likely getting the results within eight days.[83] However, colourimetric methods pose a significant bio-safety risk to laboratory staff.[65]

Microscopic-observation drug-susceptibility assay (MODS)

MODS assay is an approach for directly detecting MTB and MDR MTB in sputum or indirectly in mycobacterial broth culture. This assay uses an inverted microscope to observe MTB distinctive cord development in microscopically visible liquid media.[84] Several studies have recommended this technique as relatively quick and cost-effective for early identification of MTB and drug resistance directly from sputum specimens.[84,85] Some studies on MODS assay revealed a range between 92% to 100% of sensitivities and specificities for determining the sensitivity to each RIF, INH, and fluoroquinolone. The DST findings are obtained within seven days using this method.[86]

BACTEC radiometric method (BACTEC-460)

The BACTEC 460 (Becton Dickinson, Sparks, Maryland) is a radiometric method that detects $^{14}CO_2$ for indicating mycobacterial growth. The Bactec vials contain Middlebrook 7H12 medium and ^{4}C labelled palmitic acid substrates as the sole carbon source. When mycobacteria grow, $^{14}CO_2$ is produced as a metabolic end-product instead of CO_2. The gas is removed and analyzed by the BACTEC 460 TB SYSTEM. The radioactive ^{14}C released amount is converted to a numerical value of Growth Index (GI), which indicates a positive mycobacterial growth if it is ten or more. Although this test's sensitivity and specificity are significantly high, it uses radioactive carbon, whose half-life is 5,000 years, requiring complicated and costly disposal. The BACTEC system can be used in level II laboratories and for particularly diagnosing the cases of extrapulmonary and smear-negative TB.[71,87]

BacT/alert 3D

The BacT/ALERT® 3D System (bioMe'rieux, Marcy Etoile France) is a nonradiometric, liquid-based automated test that is developed for mycobacteria primary isolation and DST. This test is performed in a tube using a liquid emulsion sensor and a complex computer algorithm that relies on detecting carbon dioxide decrease.[88] Growing bacteria emit CO_2, which interacts with the sensor, causing a colour shift monitored by a reflectometric detecting unit housed within each incubation drawer of the instrument. Some disadvantages of the BacT/alert 3D system include susceptibility to contamination, longer turnaround time, require expensive and non-robust machine, along with the complications and hazardousness of procedures.[87]

Mycobacterial Growth Indicator Tube (MGIT)

MGIT (Becton Dickinson, Sparks, Maryland, USA) detects mycobacterial growth by

fluorescence. The test tube contains a modified Middlebrook 7H9 medium and a fluorescence quenching-based oxygen sensor at the bottom of the tube.[89] The indicator fluoresces under UV light when the bacteria grow and use oxygen, and the proliferation in the presence of the test drug reveals resistance.[90] When the growth unit (GU) in growth control hits 400, the GU values of the drugs containing tubes are observed immediately. If the GU of the drug tube is less than 100, the isolate is susceptible; if the GU of the drug tube is 100 or above, the isolate is resistant to the tested drug.[91,92] The manual and automated MGIT 960 systems have demonstrated a great connection to traditional DST techniques for rapidly identifying resistance to first and second-line anti-TB drugs.[93]

Limitations of conventional methods for TB diagnosis and drug susceptibility testing
Sputum smear microscopy is the principal diagnostic technique for detecting PTB in many high-TB-burden countries.[94] Traditional sputum smear microscopy is inexpensive and requires minimal laboratory infrastructure; competent technicians can read 20 – 30 slides daily. However, this diagnostic method has a low sensitivity (20 – 80%) and limited accuracy, particularly among HIV and immunocompromised patients.[95] Unlike traditional staining, fluorescent dyes allow for easier detection at lower magnification powers, FM is around 10% more sensitive than light microscopy.[96] However, conventional FM microscopes areexpensive and require special laboratory requirements, such as a dark room. Light-emitting diode (LED) microscopes, such as the Primo Star iLED and the CyScope® TB Fluorescence Microscope, have been developed to overcome these issues.[94]

Because various respiratory illnesses can induce X-ray deviations, chest X-rays have a high sensitivity (80 – 95%) but a low specificity (70 – 75%) for TB disease. As a result, a chest X-ray is an effective preliminary screening method that can identify suspected people who need further investigation to confirm TB. In chest radiography, automated reading algorithms have become increasingly significant. The utilization of digital radiography, as well as computer-assisted diagnostics, is revolutionizing radiographic instruments.[97] Computed radiography uses a

specific plate that produces an analogue signal, allowing the image to be digitized and stored. At the same time, direct digital radiography uses a digital detector.[98] Although traditional radiography has lower costs, digital radiography is more cost-effective in the long run because it decreases recurrent expenditures and reduces reagent consumption and radiation exposure. Furthermore, digital radiography provides more convenient data preservation and transfer, but it should be emphasized that sufficient data transmission capability is required.[94]

Mycobacterial cultures are the gold standard for TB diagnosis and DST. MTB cultures are highly sensitive and can detect low numbers of bacterial cells. However, one of their significant problems is that culture-based TB diagnosis takes a long time. In addition, skilled personnel are required to process and monitor cultures. Further, biosafety level 3 settings are needed for culturing specimens suspected to have MTB.[94] Compared to solid cultures, in which MTB takes a long time to grow, the liquid culture enables faster results, which may be produced within 10 to 14 days and up to 10% higher sensitivity. Moreover, the resistance to RIF, INH, PZA, EMB, and STR may all be identified using liquid cultures.[99] Notably, the WHO has recommended automated liquid culture systems for DST, including the Bactec MGIT (BD Diagnostics, New Jersey, USA), mycobacterial growth indicator tube, scan-processed MGIT sputum bottles and BacT/Alert 3D (bioMᵢrieux, Marcy l'Etoile, France). However, liquid culture systems are more sophisticated and costly than solid culture systems and can be prone to contamination.[94,100]

Molecular-based approaches for diagnosis and detecting drug susceptibility of MTB
There are over 170 known mycobacterial species, most related to the nontuberculous mycobacteria (NTM) group, with some belonging to the MTB complex (MTBc), sharing 99.9% genomic similarity.[66] Restriction endonuclease analysis and nucleic acid hybridization were employed for TB diagnosis. These approaches provided a strategy for rapidly diagnosing TB disease by identifying particular nucleotide sequences in the organism utilizing probes of labelled nucleic acid sequences.[101,102] The FDA

Table 2. Principles and features of first-generation and next-generation sequencer

First-generation sequencer	Next-generation sequencer
• Essentially, automated electrophoresis devices that track the movement of labelled DNA fragments make up the first generation of DNA sequencers.[134] • Able to genotype genetic markers if just a DNA fragment's length is known.[136]	• Rapid data production by parallel sequencing of large volumes of DNA utilizing many techniques.[135] • Create diverse tactics that combine genomic alignment and assembly approaches, template preparation, sequencing and imaging.[137]

approved the Accuprobe MTBc test in 1990 for identifying MTBc isolated in culture. It was one of the most incredible accuracies ever reported of a molecular technique used for mycobacteria identification from the culture.[103] The DNA-labeled probes hybridize with mycobacteria's RNA, giving a stable DNA-RNA construction. These hybrids are detected by light emission on a luminometer after adding a selection component to distinguish hybridized probes from non-hybridized ones.[104]

Phenotypic drug susceptibility testing requires mycobacteria to be in an active growth phase; thus, results are obtained within at least two to three weeks if liquid culture media is utilized and up to four to eight weeks when grown on solid media.This process will delay the patient's best therapy choice. Consequently, delayed results by conventional methods will likely contribute to acquiring further drug resistance and spreading drug-resistant MTB strains; thus, quick and dependable techniques for detecting drug-resistant TB are essential. The progress in molecular biology techniques and the knowledge of drug resistance on the molecular levels have provided advanced approaches for rapidly detecting resistance in MTB. The molecular-based approaches, detecting changes in genes coding for anti-TB drug resistance, could provide results within 24 to 48 hours.[94,99,101]

The clinical use of NAATs and whole-genome sequencing (WGS) has grown remarkably in recent years. However, owing to the high cost of WGS (Table 2), NAATs are the most commonly used. Indeed, several laboratories routinely employ NAAT-based methods for species and drug resistance identification from cultures or directly from specimens. The ribosomal 16S rRNA-targeted PCR followed by DNA sequencing is a gold standard for detecting MTB.[105] The 16S rRNA includes species-specific hypervariable areas

that benefit specific species identification. Other targets include the 16S-23S rRNA intragenic region and anti-TB drug resistance genes, including *rpoB*, *gyrB*, *hsp65*, *recA* and *sodA*.[106,107]

Collectively, the sensitivity, specificity and capability to precisely identify MTB infections are all improved by molecular-based techniques. Several molecular-based methods have been developed for diagnosing TB and determining drug susceptibility, which have sped up turnaround times and allowed point-of-care testing.[18] The following sections will outline the available molecular tests for TB diagnosis and determination of MTB drug susceptibility, addressing their benefits and limitations.

Molecular methods used for the identification of MTB
Nucleic acid amplification tests (NAATs)

The NAATs have been utilized as a practical alternative since the traditional phenotypic diagnosis of TB has several problems.[108] Because of the NAAT systems' quick turnaround times, testing and treatment can be started in the same visit, thus reducing the number of patients lost to follow-up.[109] Most NAATs identify the MTB complex bacteria, which consists of a group of closely related species, by detecting the mycobacterial insertion element IS6110 (Table 3).[110]

The AFB smear-positive and AFB smear-negative sputum specimens can both have MTB ribosomal DNA detected by NAATs.[111] The NAATs demonstrated high sensitivity in patients with positive sputum smears and between 61% and 76% sensitivity in those with negative sputum smears.[109] The GeneXpert/RIF MTB test is the NAAT system approved by the WHO.[112] The other NAATs, Amplicor *M. tuberculosis* Test (Roche. Molecular Systems, Inc) and Amplified *M. tuberculosis* Direct (MTD; Gen-Probe, Inc), were approved by the

Table 3. Main characteristics of the molecular assays used for the detection of drug-resistance-associated mutations in *M. tuberculosis*

1-Line probe assay (DNA•STRIP technology)

Test specification	Manufacturer	Detection Target	Mechanism	Time	Advantages and Limitations	Ref.
INNO-LiPA Rif TB Assay	Innogenetics, Zwijndrecht, Belgium	MTBs and rpoB for RIF resistance	It has 10 probes identifies MTBc, 5 specific regions of the rpoB gene in wild types, and 4 detect mutations	2 to 3 days	- It accurately detects resistance to RIF - Less sensitive for the detection of M. tuberculosis complex - Less sensitive when applied to clinical specimens	[185]
GenoType MTBDRplus assay VER 1.0 and VER 2.0	Hain LifeScience GmbH, Germany	MTBs rpoB for RIF resistance, katG and inhA for INH resistance	- 12 probes for rpoB gene (8 for wild type and 4 for mutant type) - 3 probes for katG INH (1 for wild type and 2 for mutant type) - 6 probes for inhA promoter (2 for wild type and 4 for mutant type)	2 to 3 days	- It is highly sensitive and specific for early detection of MDR-TB. However, - The diagnostic performance of this molecular assay in direct smear negative sputum sample is low and showed a high level of invalid results for the detection of M. tuberculosis and its resistance to RMP and/or INH	[104, 186]
GenoType MTBDRsl assay VER 1.0 and 2.0	Hain LifeScience GmbH, Germany	- MTBs - rss gene for SLID, - gyrA gene and gyrB gene for FQs - embB gene for EMB (IN GenoType MTBDRsl assay VER 1.0 ONLY) - eis promoter for KAN SLID (second-line drugs)	Each strip of GenoType MTBDRsl assay VER2.0 has 9 probes for gyrA, 3 probes for gyrB 4 probes for rss 4 probes for eis promotor	48 h	- GenoType MTBDRsl VER2.0 had variations in the overall specificity and sensitivity for identifying XDR isolates. - Additionally, results for smear-negative sputum samples were difficult to interpret	[106, 187, 188]

Table 3. Cont...

Test specification	Manufacturer	Detection Target	Mechanism	Time	Advantages and Limitations	Ref.
1-Line probe assay (DNA•STRIP technology)						
AID TB Resistance LPA	Aid Diagnostika GmbH	MTBs rpoB for RIF resistance, katG and inhA for INH resistance rpsL and rrs 500 region for STR rrs 1400 region for SLID, gyrA for FQ. embB for EMB	6 probes for rpoB 2 probes for katG 2 probes for inhA 5 probes for rpsL and rrs 500 region fo 5 probes for rrs 1400 region 7 probes for gyrA 5 probes for embB	48 h	- All AID modules have moderate sensitivity and reasonable specificity for the detection of M. tuberculosis in sputum samples - However, the high proportion of invalid tests, especially concerning resistance testing, is problematic and hampers the evaluation	[187, 189]
Nipro NTMC/MDR TB	Nipro Co., Osaka, Japan	MTBs rpoB for RIF resistance, katG, and inhA for INH resistance	9 probes for rpoB 6 probes for katG 2 probes for inhA	24 h	Good sensitivity and specificity for detection of MDR-TB in smear-positive samples only	[107]
2- Real-time PCR						
The Xpert MTB/RIF	Cepheid, Sunnyvale, California, USA	Semi-quantitative nested real-time PCR (Molecular beacon) -MTBs -rpoB for RIF resistance	Amplify the rpoB gene's 81 bp hotspot region.	2 h	- It is a completely automated technology -High sensitivity and specificity for smear-positive samples but - Moderate sensitivity and specificity for smear-negative samples - Inability to test for and detect INH resistance - High cost - False-positive findings owing to silent mutations	[159]
Xpert MTB/RIF Ultra system	Cepheid, Sunnyvale, California, USA	Semi-quantitative nested real-time PCR (Molecular beacon) -MTBs -rpoB for RIF resistance	As a replacement for Xpert MTB/RIF due to its greater sensitivity in detecting MTB and omitting the silent rpoB mutations Q513Q and F514F	Less than 90 min	- Improve detection of MTB complex and rpoB silent mutations - Low specificity in patients from high-incidence countries INH resistance - Inability to test for and detect	[163]

Table 3. Cont...

Test specification	Manufacturer	Detection Target	Mechanism	Time	Advantages and Limitations	Ref.
1-Line probe assay (DNA•STRIP technology)						
The Genedrive MTB/RIF ID Kit	Epistem, United Kingdom	Asymmetric real-time PCR (Highlighter Probes) MTB and RIF-resistant TB	Detects mutations in a rpoB 81-bp hotspot area of at codons 516, 526, and 531	Less than 1 h	- Fast results and cheap cost - Accessible to low-income communities - Inability to test for and detect INH resistance - Do not accommodate all RIF mutations	[39, 165, 191]
Abbott RealTime MTB RIF/INH	Abbott-RIF/INH	Real-time PCR	rpoB for RIF resistance and katG and inhA upper promoter region for INH	2 h	Good assay for the diagnosis of TB and DR-TB	[166, 192]
Anyplex II MTB/MDR/X DR	(Seegene, South Korea)	Multiplex real-time PCR dual-priming oligonucleotides and tagging oligonucleotide cleavage and extension for detection of RIF, INH, SL, FQ resistance	18 mutations in rpoB 7 mutations in the katG and inhA promoters, 7 mutations in gyrA 3 mutations in the rrs and eis promoters	2-3 h	- High specificity and moderate sensitivity for detection of MTB and drug resistance to TB	[67, 192]
FluoroType MTBDR	Hain Lifescience, Nehren, Germany	-Linear-after-the-exponential PCR (LATE-PCR) with probes using lights-on/lights-off detection technique. - For the detection of RIF, INH resistance	Can detect rpoB mutations, katG mutations, and inhA mutations in one single tube.	up to 96 samples in a closed system within 3-4 h from DNA extraction	- High sensitivity and specificity for the detection of rifampin and isoniazid resistance less hands-on hours, quicker outcomes, automated interpretation -The lower possibility of DNA contamination	[169, 170]
BD MAX MDR-TB assay (BD MAX)	Becton, Dickinson and Company, New Jersey, USA)	Real-time PCR TaqMan probes for the detection of RIF, INH resistance	rpoB and katG genes and the inhA promoter	4 h for testing 24 samples	- High sensitivity and specificity for the detection of RIF and INH resistance in short time - It does not identify new mutations and the sample needs to be sequenced.	[173]

EMB, ethambutol; FQ, fluoroquinolones; INH, isoniazid; KAN, kanamycin; MDRTB, multidrug-resistant tuberculosis; MTB, M. tuberculosis; MUT, mutation; NTM, nontuberculous mycobacteria; RIF, rifampicin; SLID, second-line injectable drugs; STR, streptomycin; TB, tuberculosis; h, hour

FDA for evaluating respiratory AFB smear-positive samples.[113-115] The available NAATs include the *M. tuberculosis* detection device based on loop-mediated isothermal amplification, the cross-priming amplification-based TB diagnostic system, and the Genedriv® *Mycobacterium tuberculosis* iD.[116-118]

A positive AFB smear with a positive NAAT would suggest active TB in areas with multibacillary illnesses with a high mycobacterial burden. Still, a positive AFB smear with a negative NAAT without inhibitors would indicate nontuberculous mycobacterial (NTM) disease.[119] If the culture in this situation showed positive, the doctor might classify the patient as having TB.[120] NAAT might be used for patients suspected of MTB infection with an AFB smear-positive to assess their TB stage.[121] Significantly, starting anti-TB medication would depend on the clinical decision while awaiting culture findings if the NAAT test is positive, and the result of the AFB smear is negative.[122] Centres for Disease Control (CDC) recommends testing other samples with NAAT if the sputum is smear-negative and the NAAT is also negative.[123] However, if the culture shows MTB growth, the patient might be considered to have PTB. The NAAT method for diagnosing TB is shown in Figure 3 and follows the WHO and the CDC recommendations.[124] Limiting the NAAT to cases with positive smear findings may be more effective in areas with low rates of TB cultures. On the other hand, a NAAT should be employed in patients with negative smear results in high TB rates areas.[125] According to a meta-analysis of 125 trials, NAATs should not replace standard tests for diagnosing PTB.[126]

NAAT-based TB diagnostic device: GeneXpert MTB/RIF assay

The real-time PCR (RT-PCR)technology generally uses fluorescent TaqMan or Molecular Beacons probes for concurrent target amplification and detection. The probes have a fluorochrome and an inhibitor that is closely spaced from one another. As a result, separation occurs when a link forms with the amplified target and the emitted light is detected, equivalent to the quantity of DNA in the sample.[66] This approach has the advantage of quantification of the microorganism in the clinical sample, in addition

to a lower risk of contamination and a quicker turnaround time compared to Line probe assays (LPAs) due to the removal of the hybridization phase.[127] The sensitivity and specificity of RT-PCR for smear-positive TB cases are 100% and 99 %, respectively, while for smear-negative cases are 67% and 99%, respectively.[128] RT-PCR, however, has certain limitations, as it needs costly, specialized equipment, skilled personnel, and an amplified target size range to identify multiple mutations effectively.[127]

The GeneXpert MTB/RIF assay is based on NAAT, particularly RT-PCR, with results available within two hours. It is a completely automated technology that requires no human intervention other than inserting the sample into the cartridge.[129] GeneXpert MTB/RIF test was first suggested in 2010 for adult sputum specimen-based diagnosis of PTB.GeneXpert MTB/RIF test can identify MTB-specific DNA sequences in sputum samples.[130] More than 80% of smear-negative and 99% of smear-positive TB patients could be diagnosed by a single GeneXpert MTB/RIF test performed directly using the sputum sample.[131,132] GeneXpert MTB/RIF assay can be utilized as an additional test after microscopic MTB examination, replacing AFB smear microscopy and identifying MTB in both AFB smear-positive and smear-negative culture-positive cases. It can also detect MTB in pleural fluid, a lymph node biopsy or fine-needle aspiration, gastric juice, cerebrospinal fluid and tissue samples. Still, GeneXpert MTB/RIF assay can be used as the initial test to diagnose PTB-suspected people.[133]

The GeneXpert MTB/RIF test can be utilized as an initial or a further test after a negative AFB smear microscopy result.[134,135] Notably, despite the high cost, the median time to therapy for AFB smear-negative TB has decreased from 56 days (range 39 - 81 days) to 5 days (range 2 - 8 days) owing to the GeneXpert MTB/RIF test implementation.[136,137] However, the quick sputum-based test would be more cost-effective than the traditional sputum smear microscopy.[138,139] Although the proficiency in lowering the early missing to follow-up patients and shortening therapy period commencement, the introduction of GeneXpert MTB/RIF would not be able to enhance controlling drug-resistant TB.[140]

Whole-genome sequencing (WGS) as a diagnostic approach for TB

The investigation of genetic markers of organisms for diagnosis, therapy and follow-up infection prognosis has become possible through advances in microbial genomics.[141] As MTB lineages spread among people, whole-genome sequencing (WGS) is developing into an accessible and inexpensive technique for detecting microevolution within those lineages.[142] Figure 4 shows the outlines of the WGS process from the specimen collection to the diagnostic report. There are two DNA sequencers: first-generation and second-generation sequencers (widely known as the next-generation sequencer [NGS]). The first-generation sequencer has a low cost (about $65 per bacterial genome) and remarkable throughput but relative slowness. The second generation can sequence many genomes in less than a day but has a lower throughput and higher cost (the IlluminaR MiSeq costs around $150 per genome).[143] Table 2 describes the principles of first-generation and next-generation DNA sequencing techniques.

Molecular-based determination of drug susceptibility of MTB

TB has become resistant to clinically used drugs to cure the disease. MTB strains likely gain drug resistance during patient treatment because of delayed diagnosis, unsuitable medicine, or poor adherence to the treatment regimen. Thus, the rapid detection of TB helps timely access to the proper treatment, decreases transmission rates and improves treatment outcomes.[100] The progress in molecular biology techniques and knowledge of drug resistance on the molecular levels have provided advanced approaches for rapidly detecting drug resistance in MTB.[144,145]

Single nucleotide polymorphisms (SNPs) within the MTB chromosome are the primary means by which MTB strains develop drug resistance owing to insertions or deletions events.[146,147] Mutations in one or more genes have been identified for anti-TB drugs. Moreover, each mutation is associated with varying levels of drug resistance. For example, *rpoB* gene alterations, predominantly in an 81 bp hotspot area, are responsible for 97 % of resistance to RIF. Mutations in the *katG* and *inhA* genes or *inhA* promoter region cause MTB to acquire resistance

to INH.[148] RIF resistance in MTB can be considered a surrogate marker for MDR strains; consequently, the molecular-based method should detect the mutations leading to RIF resistance, which indicates MDR TB.[149,150]

In 2020, the WHO recommended using molecular assays for rapidly diagnosing MDR TB.[151] Many molecular-based techniques have been developed to simultaneously identify MTB and its drug resistance to RIF and INH. These assays detect resistance-related mutations in genes encoding resistance of MTB isolates. In addition, they have the advantage of yielding results in one to two days and may be employed directly for smear-positive sputum and other clinical specimens.[152] Therefore, several highly sensitive and specific nucleic acid-based assays that identify mutations linked to resistance to antitubercular drugs have been designed and developed; thus, that allows for low-cost, reliable, straightforward, and quick results despite the high-cost laboratory infrastructures (Table 3).[153]

Solid-phase hybridization or line probe assays

Line probe assays are a group of modern DNA strip tests that utilize PCR and reverse hybridization assays. These tests include several steps: extracting DNA from both mycobacterial isolate or clinical specimens, then PCR-based nucleic acid amplification and hybridizing PCR products with oligonucleotide probes immobilized on a strip. Completely, hybridization is indicated by developing a coloured reaction on the strip as lines where the probes are located (thus, the term "line-probe").[100,154]

The commercially available solid phase reverse hybridization assays include the Line Probe Assay (LiPA) (INNO-LiPA Rif TB Assay, Innogenetics, Zwijndrecht, Belgium) for identifying MTB and screening for RIF resistance. INNO-LIPA has ten probes on its strip, one identifying MTBc, five highlighting specific regions and four detecting mutations of the *rpoB* gene. It showed high sensitivity when used on mycobacterial culture but less in direct specimens. Only the *rpoB* flashpoint area of mutations (codon 509 to codon 534; Asp516Val, His526Tyr, His526Asp, and Ser531Leu) is examined.[155] A meta-analysis suggested that the LiPA assay is highly sensitive and specific for detecting RIF-resistant MTB. In this analysis, 12

of 14 published studies showed a sensitivity> 95% with a specificity of 100% when the assay was employed on isolates but was less sensitive when applied to clinical specimens. However, four studies on clinical samples revealed 100% specificity, but the sensitivity ranged from 80% to 100%.[156] However, another study that evaluated LiPA for determining RIF resistance in 420 sputum samples revealed 99.6% agreement between culture-based identification and LiPA. The study addressed that with satisfactory DNA extraction, LiPA allows rapid detection of resistance to RIF when employed directly on sputum.[157]

AID TB Resistance LPA (Aid Diagnostika GmbH) is used to identify resistance to first- and second-line anti-TB drugs in clinical specimens and culture via three modules. Module 1 detects *rpoB, katG*, and *inhA* promoter genes; module 2 examines *rpsL* and *rrs* to identify aminoglycoside resistance (STR, AMK, CAP), and module 3 examines *gyrA* and *embB* to find FQ and EMB resistance.[158] The three modules comprise both wild-type and mutant probes. The AID TB Resistance LPA showed remarkable specificity and sensitivity for detecting resistance to RIF, INH, STR, FQs, and second-line injections. (ranged from 90% to 100%), but with reduced sensitivity to detect resistance to EMB of 72.9 %.[159]

WHO suggested GenoType MTBDR*plus* assay (Hain LifeScience GmbH, Germany) and Nipro NTMCMDRTB (Nipro Co., Osaka, Japan) for the early screening for drug resistance in sputum smear-positive samples.[160] The GenoType MTBDR*plus* assay identifies *rpoB* mutations in addition to INH resistance by examining the *inhA* gene (which encodes low-level resistance) and the *katG* gene (which encodes high-level resistance). It has probes for wild-type areas of susceptible strains and their associated mutations. WHO approved GenoType MTBDR*plus* assay in 2008 and may be utilized on clinical samples directly or cultures with high sensitivity and specificity for RIFresistance (88.2% and 89.5%, respectively) and INHresistance (91.7 and 97.2%, respectively). It delivers results in 48 to 72 hours.[161,162]

Several studies performed on MTBDR*plus* VER2.0 assay have shown high sensitivity (83.3% to 96.4%) and specificity (98.6% to 100%) for detecting MDR isolates in smear-positive specimens.[100,162] GenoType MTBDR*sl* VER2.0 can detect resistance to second-line anti-TB drugs such as CP, AK, and KM (*rss* gene), fluoroquinolones (*gyrA* and *gyrB* genes), and KAN (*eis* promoter gene).[66] It has variable sensitivity and specificity when screening for resistance to second-line injectable drugs. Still, it has excellent sensitivity and specificity (91% to 100%) for identifying resistance to fluoroquinolone.[163] Although; there are variations in the overall specificity and sensitivity of GenoType MTBDR*sl* VER2.0 for identifying XDR MTB isolates. Additionally, results for smear-negative sputum samples were difficult to interpret.[104] In addition, Nipro NTMCMDR-TB detects MDR-TB by targeting the genes *rpoB, katG,* and *inhA* and can differentiate four important *Mycobacterium* species (MTB, *M. avium, M. intracellulare,* and *M. kansasii*) that cause diseases in humans.[164] Solid-phase hybridization assays are reasonably simple, rapid and straightforward. Yet, fundamental expertise in molecular-based methods is necessary. In addition, the test sensitivity varies with the amount of DNA in the sample, and the presence of inhibitors might lead to false-negative results. Furthermore, LPA methods target only the primary mutation; thus, the specificity and sensitivity may vary if mutations happen in target regions.[160]

Multiple allele-specific PCR (MAS-PCR)

MAS-PCR can concurrently detect common mutations in the RIF resistance gene, thus reducing the cost and practical procedures. MAS-PCR aims to detect RIF resistance-conferring mutations in codons 435, 445, and 450 of the *rpoB* gene using pure DNA extracted from mycobacterial culture.[165,166] These point mutations have been detected according to the wild-type sequences of strain H37Rv. The 3 prime ends of allele-specific primers pair with the nucleotide base of the respective codon. If the investigated strain carries the targeted position as the wild-type, the allele-specific region can be amplified by PCR to produce a visible DNA fragment. In contrast, if the target DNA sequence contains a mutation, it will block the PCR amplification, giving no DNA band.[165] In a study evaluating the effectiveness, MAS-PCR showed a sensitivity of 88.3% and 100% specificity compared to the DST by proportion method.[166] In another study, the three RIF resistance-related mutations were identified by MAS-PCR with

97.9% sensitivity and 100% specificity compared to the standard DST. MAS-PCR could be a suitable method for routinely detecting RIF-resistant MTB, providing a fast, cost-effective and straightforward method.[167]

RT-PCR-based techniques for detecting drug resistance

The GeneXpert MTB/RIF (Cepheid, Sunnyvale, California, USA) is recommended by WHO as a rapid molecular test for identifying MTB complex and screening for RIF resistance in MTB isolates.[160] The Xpert MTB/RIF test employs semi-quantitative nested RT-PCR technology to detect the *rpoB* gene 81 bp hotspot region to identify mutations linked to RIF resistance.[168,169] The WHO recommended this approach as the primitive diagnostic step in high-risk regions of MDR TB.[170] In addition, Gonnalves et al. study findings confirmed that RT-PCR detects RIF resistance with a sensitivity of 99% and specificity of 100% in less than four hours.[171] However, some studies reported false-positive results by GeneXpert MTB/RIF owing to silent mutations and false-negative results because it was incapable of identifying RIF resistance-related mutations beyond the hotspot region.[168,172]

The next-generation Xpert MTB/RIF Ultra system (Cepheid, Sunnyvale, California, USA) includes two more targets for MTB complex identification (IS1081 and IS6110) with a tenfold improvement in analytical sensitivity.[173] Xpert MTB/RIF Ultra undertakes Xpert MTB/RIF limitations by omitting silent *rpoB* mutations Q513Q and F514F.[174] The next-generation Ultra system was recommended as a first diagnostic test for all patients, whatever the age, with TB symptoms and for testing extrapulmonary specimens, including tissue and lymph node samples and cerebrospinal fluid.[160]

The Genedrive MTB/RIF ID Kit (Epistem, UK) can detect MTB and RIF resistance in MTB from raw sputum samples. The system employs a straightforward paper-based DNA extraction process, asymmetric RT-PCR, and a patented hybridization probe technology (Highlighter Probes).[175] The system has an overall sensitivity of 72.3 % for *rpoB* mutation detection, as it can detect the mutations in the 81-bp hotspot area of *rpoB* at codons 516, 526, and 531. This system has

the advantage of a short time round and low cost; thus, it is accessible to low-income communities.[93]

The Abbott RealTime MTB RIF/INH Resistance can detect resistance to MTB and RIF by targeting *rpoB* and INH resistance genes by targeting *katG* and *inhA* upper promoter region for INH. The sensitivity of detecting each RIF and INH resistance is 100% and 94.3%., respectively, and the specificity is 100% and 94.3%, respectively. It was configured as a companion assay for the Abbott Real-time MTB assay.[176] Both assays are used on the high-throughput automated Abbott m2000 system and can handle three to 96 samples in one run, with controls and specimens.[177]

Anyplex II MTB/MDR/XDR (Seegene, South Korea) is a multiplex RT-PCR that detects MTBc and resistance to RIF, INH, FQs, and injectable drugs. Anyplex is programmed to identify 18 mutations in *rpoB*, seven mutations in the *katG* and *inhA* promoters, three mutations in *gyrA*, and three mutations in the *rrs* and *eis* promoter regions, which cause resistance to RIF, INH, FQs, and aminoglycosides, respectively. This test depends on two techniques that aid in identifying particular mutations in target genes: dual-priming oligonucleotides and tagging oligonucleotide cleavage and extension. Specificity was between 94% and 100%, while sensitivity ranged between 50% and 100% to detect MTB and drug resistance.[72]

FluoroType MTBDR (Hain Lifescience, Nehren, Germany) is a new assay with a different technology. This test incorporates the linear-after-the-exponential PCR (LATE-PCR) with probes using the Lights-on/lights-off technique.[178] One tube can detect the *rpoB*, *katG*, and *inhA* regulatory region mutations in respiratory and non-respiratory clinical samples. The absence of a wild-type band may indicate other mutations within the amplified region of the target genes. The test is performed in a FluoroCycler96 instrument (Hain Lifescience), allowing testing of up to 96 samples within three to four hours from DNA extraction. Melting curves are the data of the FluoroType MTBDR, and the shapes indicate either wild types or the existence of definite mutations.[179] Compared to the Genotype MTBDRplus, the FluoroType MTBDR provides several advantages: fewer hands-on hours, quicker outcomes, automated interpretation with the capability of instantly importing findings into a

lab information system, and a lower possibility of DNA contamination. FluoroType MTBDR showed excellent sensitivity and specificity for determining RIF and INH resistance when using culture isolates. A study reported the sensitivity and specificity of FluoroType MTBDR to be 91.7% and 97% for INH, respectively, and 98.9% and 95.6% for RIF, respectively. Another study revealed sensitivity and specificity as 98.9% and 100% for RIF, respectively, and 98.8% and 100% for INH, respectively.[180] A recent study detected sensitivity and specificity for RIF resistance in smear-positive specimens of 100% and 97.8%, respectively, and in smear-negative samples of 100% and 96.9%, respectively. As for INH, both detection sensitivity and specificity were 100% in smear-positive specimens and 93.8% and 97.4% in smear-negative samples, respectively.[181]

BD MAX MDR-TB assay (BD MAX) (Becton and Dickinson, 2018) is an automated, qualitative diagnostic test for the direct detection of MTBc DNA and mutations in *rpoB* and *katG* genes and the *inhA* promoter area in sputum from patients with clinical suspicion of TB disease.[182] The assay employs RT-PCR of particular DNA targets and fluorogenic target-specific hybridization probes. The assay is fully automated and requires a steady electricity supply and laboratory worker training. The pre-validation study of the assay demonstrated high sensitivity and specificity similar to the Abbott RealTime MTB RIF/INH Resistance assay and the FluoroType MTBDR for detecting MTBc DNA and mutations associated with resistance to RIF and INH.[183] A recent multicenter study performed on 1053 participants with presumptive TB reported that the sensitivity and specificity of BD MAX MDR-TB for RIF resistance compared with phenotypic DST were 90% and 95%, respectively. Sensitivity and specificity for detecting INH resistance were 82% and 100%, respectively.[173] It has a faster turnaround time than the GeneXpert MTB/RIF, i.e., four hours for testing 24 samples, compared to the GeneXpert MTB/RIF TAT, which takes around 2 hours for testing one sample. However, like other molecular assays, it does not recognize new mutations, and thus the sample needs sequencing.[184]

Droplet digital PCR-based technique (DDPCR)

Pholwat et al. developed a digital PCR-based method to detect and quantify various resistance subpopulations in a mycobacterial community containing even one XDR-MTB among thousands of susceptible MTB, i.e., heteroresistance.[185,186] DDPCR combines microfluidic technology and PCR, enabling precise quantification of target DNA with high sensitivity and specificity.[162] DDPCR can split the sample into thousands of drops and run PCR for individual sub-reactions with little or no off-target sequence in each sub-reaction.[187] The fluorescence signal is detected in each droplet following the PCR procedures. Poisson statistical analysis is used for positive droplets to quantify the target sequence accurately. The PCR reaction setup and amplification are identical to quantitative PCR, except some contain the DNA target while others do not. This reproducible method detects bacilli at a count as low as 1000 CFU/ml. With these benefits, DDPCR enables the early detection of emerging mutations that evolve in treatment and may necessitate a medication adjustment.[185]

DNA sequencing-based identification of drug resistance

DNA sequencing is considered the gold standard molecular approach because it visualizes the nucleotide sequence of the target DNA position in a short time (10 to 12 hours). As in INH resistance, many genes may be involved in a single drug resistance, or the mutations may be distributed across a large gene segment. On the other hand, PCR-based sequencing (PCR amplification followed by gene sequencing) is a suitable approach for particular applications such as investigating *rpoB* hotspots, where mutations linked with RIF resistance are localized in a concise portion of the gene. However, unfortunately, sequencing is not as simply applicable for routine diagnosis of TB and identification of drug resistance-related mutations.[55]

Developing Next Generation Sequencing (NGS) has enabled bacterial species identification, showing all (known and novel) mutations, including synonymous and non-synonymous mutations, insertions and deletions in a sample, determination of antimicrobial resistance profile and so optimal therapy, and prediction of organism evolution.[188,189] The AmpliSeq for Illumina TB Research Panel (Illumina) amplifies

and sequences full-length of eight genes (*rpoB*, *kat*G, and *inhA*, *pncA*, *gyrA*, *eis*, *embB,* and *rpsL*), not of entire genomes. Continuous advances in whole genome sequencing (WGS) techniques and bioinformatics analysis software have extended the chances of follow-up drug resistance in TB. Thus, it is possible to accurately detect resistance within one to three days after sample collection to most antituberculosis drugs used for treatment based on the quality and quantity of extracted mycobacterial DNA and the sequencing platform. Unlike conventional molecular assays that detect specific targets, WGS offers detailed and precise sequencing information for whole genomes.[190]

Several researchers reported that WGS showed good sensitivity and specificity in determining resistance to the first-line drugs RIF and INH; nevertheless, there was a significant difference in WGS accuracy for the other first- and second-line drugs.[191-193] A recent study revealed that WGS has a 20% advantage over traditional genotypic techniques in finding drug resistance outside the suspected drug resistance-related region.[194] Direct sequencing from sputum samples will enable rapid and complete resistance profiling in a therapeutically relevant time frame. However, direct sputum DNA extraction presents significant obstacles due to the restricted number of bacilli in sputum, particularly in smear-negative TB cases, resulting in a poor yield of genomic DNA accessible for sequencing. Furthermore, the results varied due to the high frequency of human genome contamination.[153] A study by Colman et al. evaluated the performance of direct WGS and automated data analysis for drug resistance prediction in sputum tuberculosis samples (the Next Gen- RDST assay). The assay demonstrated high sensitivity and specificity (above 95 %) for detecting resistance to RIF, INH, and KM; still, low sensitivity in detecting resistance to amikacin, capreomycin, and fluoroquinolones compared to the phenotypic DST.[194]

Compared to the Xpert® MTB/RIF assay, the WGS is more accurate in identifying various gene mutations. Moreover, WGS disallowed false positive results in detecting the polymorphism in the rifampicin-resistance determining region (RRDR) of *rpoB*.[195] However, because the workflow to extract DNA requires MTB culturing, which takes several weeks before an appropriate amount of DNA can be recovered, WGS has not been employed as a usual TB diagnostic method.[143,153] Although one mL of early-positive MGIT cultures can be used to reliably and reasonably extract DNA for WGS to identify mycobacterial species and know its drug resistance.[196] A technique that enables WGS without prior specimen culturing has recently been developed. The method uses biotinylated RNA baits unique to MTB DNA to extract entire MTB genomes from uncultured sputum samples.[197] WGS data are collected several weeks before DST results are available.[198] DNA sequencing might be utilized to confirm RIF resistance identified by Xpert MTB/RIF.[199]

The regular WGS for diagnosis is only available in high-income nations due to the high costs, which can reach hundreds of dollars.[160,190,200] The use of WGS on a broad scale, particularly in middle- and low-income nations, remains problematic for the following reasons: the lack of standardization in WGS analysis directly from sputum, including specific procedures to remove non-mycobacterial genetic material and enrich the genome of *M. tuberculosis*, software and database must be established of this technology in this area of medicine; technical personnel and bioinformatics facilities are required, data acquisition and analysis are needed to determine whether new mutations confer anti-TB drug, in addition to the high cost of this technology.[189,201,202]

CONCLUSION

MDR TB is an infectious bacterial disease caused by MTB, one of the deadliest contagious illnesses worldwide. The inappropriate therapy contributes to the development of drug resistance in MTB, leading to a reduction in available treatments and an increase in the mortality rate. Thus, for better treatment and management, an early, quick, and accurate diagnosis of TB and determination of the antimicrobial susceptibility of the infecting strain are essential. However, clinical diagnosis is usually insufficient as MTB infection is commonly asymptomatic. In addition, the conventional culture procedures for bacterial isolation, identification and drug susceptibility testing are time-consuming and require specialized laboratories and well-trained personnel due to MTB sluggish growth. Molecular-based

technologies have sped up turnaround times after sample collection, which could enhance the control of drug-resistant TB. Although molecular-based diagnostic methods have high sensitivity and specificity, they require high-cost laboratory infrastructures and skilled personnel to perform tests. However, despite their several drawbacks, traditional laboratory techniques for TB diagnosis are still widely utilized because they are feasible, trustworthy, and cost-effective. In addition, phenotypic susceptibility testing is still required to determine its drug susceptibility. Therefore, nucleic acid amplification tests (NAATs), culture-based procedures and acid-fast smear microscopy are currently employed for the definitive laboratory diagnosis of TB.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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