Diagnosis and Management of Tuberculosis

Chapter 6

Tuberculosis: New Laboratory Diagnostic Methods and Treatment

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1. Introduction

Tuberculosis (TB) still remains one of the world's deadliest communicable diseases. The World Health Organization (WHO) estimated that there were 10.4 million new TB cases worldwide and 1.4 million TB deaths in 2015. People living with HIV accounted for 1.2 million (11%) of all new TB cases [1]. Because of the slow growth rate of the causative pathogen *Mycobacterium tuberculosis*, isolation, identification, and drug susceptibility testing (DST) of this organism can take several weeks or longer. In recent years, many new laboratory diagnostic methods have been developed for direct detection and susceptibility testing of TB. A good understanding of their effectiveness and limitations is important to improve TB diagnosis.

M. tuberculosis is a tough and resilient microorganism that is well adapted to prolonged residence in its human host. Shielded by a waxen cell wall that protects against lethal enzymes and other deadly products elaborated by the body's antibacterial defenses, tubercle bacilli are also sheltered against foreign chemicals such as gold, arsenic, mercury, calcium, iodine, quinine, creosote, turpentine, cod liver oil, and chaulmoogra oil, to mention just some of the many

"therapeutic" substances of historical interest that had been tried in a fruitless effort to arrest or reverse the progress of consumption. The goals of tuberculosis treatment are to ensure cure without relapse, to prevent death, to stop transmission, and to prevent the emergence of drug resistance. *M. tuberculosis* can remain dormant for long periods. The number of tubercle bacilli varies widely with the type of lesion, and the larger the bacterial population, the higher the probability that naturally resistant mutants are present even before treatment is started. Even up to now, treatment of tuberculosis is still a challenge work.

2. New Laboratory Diagnostic Methods of Tuberculosis

2.1. Xpert MTB/RIF assay

Xpert MTB/RIF is a new self-contained and cartridge-based assay based on the GeneXpert multi-disease testing platform, that dramatically simplifies molecular testing by fully integrating and automating the three processes required for real-time PCR-based molecular testing: sputum processing, DNA extraction and amplification, TB and drug-resistant TB diagnosis. It has similar sensitivity to culture, targets *M. tuberculosis* specifically and enables simultaneous detection of rifampicin resistance via the *rpo*B gene [2]. Xpert MTB/RIF is a simple, rapid, automated molecular assay. The 1-step external sample preparation is extremely simple. The closed system ensures that there is no risk of contamination and no requirement for bio-safety facilities. Test results can be obtained in just 90 minutes.

After completion of development in 2008, the diagnostic accuracy of the Xpert MTB/RIF assay for pulmonary TB, done by the Foundation for Innovative and New Diagnostics (FIND), were assessed in 1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary TB in Peru, Azerbaijan, South Africa and India. The result showed that among culture-positive patients, a single, direct Xpert MTB/RIF test identified 98.2% of patients with smear-positive TB and 72.5% with smear-negative TB. The test was specific in 99.2% of patients without TB. Among patients with smear-negative, culture-positive TB, the addition of a second Xpert MTB/RIF test increased sensitivity by 90.2%. As compared with phenotypic drug-susceptibility testing, Xpert MTB/RIF testing correctly identified 97.6% of patients with rifampin-resistant bacteria and 98.1% with rifampin-sensitive bacteria [3].

A recent review, which included 27 unique studies (integrating nine new studies) involving 9557 participants, indicated that as an initial test replacing smear microscopy, Xpert MTB/RIF sensitivity was 89% and specificity 99%, while as an add-on test following a negative smear microscopy result, the sensitivity was 67% and specificity was 99%. For smear-positive, culture-positive TB, Xpert MTB/RIF sensitivity was 98%. For people with HIV infection, the sensitivity was 79%, and for people without HIV infection, it was 86%. In comparison with smear microscopy, Xpert MTB/RIF increased TB detection among culture-confirmed cases by 23%. For rifampicin resistance detection, Xpert MTB/RIF sensitivity was 95% and specificity

was 98% [4].

WHO had endorsed Xpert MTB/RIF technology and released a recommendation and guidance for countries to incorporate this new technology in their programs [5]. This assay was specifically recommended for use as the initial diagnostic test for suspected drug-resistant or HIV-associated pulmonary tuberculosis. By June 2012, two-thirds of countries with a high TB burden and half of countries with a high multidrug-resistant (MDR)-TB burden had incorporated the assay into their national tuberculosis programme guidelines. In September 2013, Xpert MTB/RIF was approved by FDA [6].

The main disadvantages of Xpert MTB/RIF assay are high cost and high false positive rate in areas with low prevalence of rifampicin resistance. A recently updated WHO policy has recommended that a repeated Xpert MTB/RIF assay on a fresh specimen can be useful when it detects *M. tuberculosis* with RIF resistance in the patients considered to be at low risk of MDR-TB [7].

2.2. Line probe assay (LiPA)

LiPA is a DNA strip test combined with a reverse hybridization method that allows simultaneous molecular identification of tuberculosis and the most common genetic mutations causing resistance to rifampicin and isoniazid or the 2nd line drugs. This technology can diagnose MDR-TB or extensively drug-resistant (XDR)-TB directly from smear-positive sputum samples, providing results in just five hours - an enormous improvement on the 1 to 2 months needed for conventional DST for 1st line and 2nd line drugs.

GenoType Mycobacteria Drug Resistance (MTBDR) (Hain Lifescience GbmH, Germany) is one of the most studied LiPA commercial assays. The MTBDR assay (first version introduced in 2004) identifies mutations in the *rpo*B gene as well as mutations in the *kat*G gene for high-level isoniazid resistance [8]. The GenoType MTBDR plus, the second-generation assay, can further detect mutations in the *inh*A gene that confers resistance to low-levels of isoniazid [9]. The GenoType MTBDRsl assay can determine the genetic mutations associated with resistance to fluoroquinolone, aminoglycosides (kanamycin, amikacin), cyclic peptides (capreomycin), ethambutol, and streptomycin [10]. INNO-LiPA Rif. TB assay (Innogenetics, Belgium) is another commercialized LiPA, and also has been shown to be highly sensitive and specific in the detection of RIF-resistant *M. tuberculosis* complex (MTBC) isolates [11,12]. However, it cannot evaluate the mutations leading to isoniazid resistance [13].

Evaluation studies by FIND and others indicated that the LiPA is highly accurate in detecting MDR-TB in a variety of geographical settings, and cost-effective when compared with TB culture and DST. They also demonstrated significant patient benefits, including early targeted treatment of MDR-TB and the potential interruption of transmission. A review indi-

cated that the pooled sensitivity and pooled specificity of the MTBDRplus assay were 95.9% and 98.0%, respectively, and the sensitivity of the INNO-LiPA Rif. TB assay for rifampicin resistance was 94.1% and pooled specificity was 98.8%. Both of them maintained high negative predictive value (NPV) (greater than 95%) across prevalence rates. However, at lower prevalence rates (3%), the positive predictive value (PPV) was less than 90% for both the INNO-LiPA Rif. TB assay (71.0%) and the MTBDRplus assay (59.3%). At a higher prevalence rate of 15%, the PPV was improved, 93.3% for the INNO-LiPA Rif. TB assay and 89.2% for the MTBDRplus assay [14].

The reported sensitivity for detecting ofloxacin, amikacin, and extensive drug resistance, using the GenoType MTBDRsl, was 90.7%, 100% and 92.3%, and the specificity for detection was 98.1%, 99.4% and 99.6%, respectively. GenoType MTBDRsl revealed a significant increase in diagnostic yield of 20.1% and 19.3% for ofloxacin and amikacin resistance, respectively. In addition, implementation of this test significantly reduced the turn around time by 93.3%, calculated from the date that the specimen was received at the laboratory to reporting second-line results [15].

In 2008, the WHO issued a recommendation for the use of molecular LiPA for the rapid diagnosis of MDR-TB in high TB-burden, low-income settings. In March 2012, the Expert Group of WHO recommended that the Genotype MTBDRsl assay can not be used as a replacement test for conventional phenotypic DST. However, they noted that this technology may be used as a rule-in test for XDR-TB where line probe assay capacity is available [16]. Given high assay specificity, results could be used to guide infection control measures to interrupt transmission. Low automatization, requirement of specialized laboratory and expertise including PCR are primary disadvantages of this technique.

2.3. Loop-mediated isothermal amplification (LAMP)

LAMP is a recently developed molecular method that has been successfully implemented in the detection of *M. tuberculosis* in clinical specimens [17]. LAMP has several advantages, such as rapidity, high sensitivity, ease of application and cost-effectiveness. The test amplifies target DNA at a constant temperature, meaning that it can be carried out with minimal equipment at low-level laboratories such as those in TB endemic countries. LAMP has high specificity because of the high specificity of the target sequence, and multiple independent sequences are identified by the primers.

In April 2012, WHO convened an Expert Group to review data from field evaluation and demonstration studies on the performance of the TB LAMP assay. The Expert Group agreed that LAMP technology has potential as a rapid TB diagnostic tool but that the body of evidence presented on the assay was insufficient to make a recommendation either in favor of, or against the use of TB LAMP as a replacement test for sputum smear microscopy. The Expert

Group made several recommendations for conducting further research and studies to improve the evidence base for the technology. A multicenter study in 2016 showed TB-LAMP (Eiken Chemical Co.) sensitivities among culture-positive samples were 97.2% (243/250) and 62.0% (88/142) for smear-positive and smear-negative TB, respectively, but varied widely by country and operator. Specificities ranged from 94.5% (446/472) to 98.0% (350/357) by country. A root cause analysis identified high temperatures, high humidity, and/or low reaction volumes as possible causes for false-positive results, as they may result in nonspecific amplification [18]. Gelaw et al. assessed the diagnostic performance of LAMP assay in detecting *M. tuberculosis* infection in sputum sample compared to LED fluorescent smear microscopy. They found the agreement between the two tests was very good (kappa = 0.83, P-value </=0.0001), and LAMP showed similar specificity but a slightly lower sensitivity with LED fluorescence microscopy [19]. Nliwasa et al. found the sensitivity of LAMP was similar to Xpert MTB/RIF but lower than fluorescence smear microscopy and all three tests had high specificity [20].

2.4. Interferon gamma release assay (IGRA)

IGRA is an in vitro immune test that has been introduced in recent years for the diagnosis of tuberculosis infection. IGRA is based on the detection of a T-cell immune response towards *M. tuberculosis* complex specific antigens (culture filtrate protein (CFP)-10, early secretory antigenic target (ESAT)-6 and/or TB7.7). To date, there are two most commonly used commercial forms of IGRA: the enzyme-linked immunospot (ELISPOT)-based T-SPOT®.TB test (Oxford, UK), and the enzyme-linked immunosorbent assay (ELISA)-based QuantiFERON®-TB Gold in-tube assay (QFT-GIT) and its predecessor QuantiFERON®-TB Gold (QFT-G) test (Cellestis, Australia).

Latent *M. tuberculosis* infection (LTBI). IGRA may have a relative advantage over the tuberculin skin test (TST) in detecting LTBI and allow the exclusion of *M. tuberculosis* infection with higher reliability [21]. Both IGRA and TST have low sensitivity in a variety of immunocompromised populations [22]. IGRA were more sensitive than TST for diagnosis of *M. tuberculosis* infection in HIV-infected patients [23]. Both TST and current IGRAs primarily detecting a CD4 T-cell response have low predictive value for progression from infection to active TB [22]. However, a new generation assay, the QuantiFERON-TB Plus (QFT-Plus, Qiagen, Hilden, Germany), has been developed to stimulate IFN-γ production by both CD4 and CD8 T-cells. First results indicate that the CD8 T-cell response may be able to identify people at greater risk of progression to active TB [24].

Active TB. In blood and extra-sanguineous fluids, the pooled sensitivity for the diagnosis of active TB was 80% and 48% for QFT-GIT and 81% and 88% (confirmed and unconfirmed cases) for T-SPOT, and the pooled specificity was 79% and 82% for QFT-GIT and 59% and 82% for T-SPOT, respectively [25]. Among HIV-infected adults with active pulmonary

tuberculosis in low- and middle-income countries, pooled sensitivity were 76% for T-SPOT and 60% for QFT-GIT, and pooled specificity were 52% for T-SPOT and 50% for QFT-GIT [26].

Extrapulmonary TB (EPTB). A meta-analysis showed pooled sensitivity for the diagnosis of EPTB was 72% for QFT-G or GIT and 90% for T-SPOT, and pooled specificity for EPTB was 82% (QFT-G or GIT) and 68% (T-SPOT) [27]. A meta-analysis on pleural tuberculosis showed the pooled sensitivity and specificity for the blood assays were 77% and 71%, and for the pleural fluid assays were 72% and 78%. There was considerable heterogeneity. They concluded that commercial IGRAs, performed either on whole-blood or pleural fluid, have poor diagnostic accuracy in patients suspected to have tuberculous pleural effusion (TPE) [28]. A meta-analysis on tuberculous meningitis showed the moderate diagnostic accuracy of blood and cerebrospinal fluid (CSF) IGRA. The overall sensitivities for blood and CSF IGRAs were 78% and 77%, and the specificities were 61% and 88%, respectively [29].

Pediatric TB. Two meta-analysis studies showed the sensitivities of two IGRAs and TST in active pediatric tuberculosis were similar (70% for ELISA, 62% for ELISPOT and 71% for TST). The pooled specificity was 100% for ELISA and 90% for ELISPOT, but was much lower for TST (56% in all included studies and 49% in children with bacillus Calmette-Guerin vaccination). So IGRAs performance in children showed no better sensitivity than TST, but higher specificity [30,31].

In conclusion, current IGRAs are costly and require fairly sophisticated laboratory infrastructure and technical expertise. Their performance differs in high versus low TB and HIV incidence settings, with relatively lower sensitivity in high-burden settings. IGRAs (like the TST) cannot distinguish LTBI from active TB, and should not be used for the diagnosis of active TB disease in high-burden settings due to a high background prevalence of LTBI [32].

2.5. Urine lipoarabinomannan (LAM) test

Lipoarabinomannan is an important 17.5-kD heat-stable glycolipid found in the cell wall of *M. tuberculosis*, accounting for up to 15% of the total bacterial weight [33]. Detection of urine LAM has several advantages compared with currently used diagnostics. It is non-invasive, simple to collect, process and store, and is attractive in people with no sputum. And it has been used to develop commercially available enzyme-linked immunosorbent assays, such as Determine TB-LAM (Alere, Waltham, MA, USA) [34].

A meta-analysis in 2011 regarding use of urine LAM assays for diagnosing active TB showed sensitivity ranged from 13% to 93%, while specificity ranged from 87% to 99% in microbiologically confirmed cases, sensitivity ranged from 8% to 80%, while specificity ranged from 88% to 99% in clinical and confirmed TB cases, sensitivity was 3-53% higher in HIV-

positive than HIV-negative subgroups, and sensitivity was highest with advanced immunosuppression [35]. It suggests urine LAM assay has suboptimal sensitivity for routine clinical use. However, Lawn et al. evaluated the diagnostic accuracy of Determine TB-LAM and found it provided results within 30 min and had highest sensitivity at low CD4 cell counts: 66.7% at <50 cells per muL, 51.7% at <100 cells per muL, and 39.0% at <200 cells per muL; specificity was greater than 98%. It provides important incremental yield when combined with sputum smear microscopy, which did not differ statistically from the sensitivities obtained by testing a single sputum sample with the Xpert MTB/RIF assay. Urine LAM test is a simple, low-cost, alternative to existing diagnostic assays for tuberculosis screening in HIV-infected patients with very low CD4 cell counts [34]. Other studies also reported similar findings [36-38].

Kroidl et al. evaluated the diagnostic performance of two urine LAM tests (MTB-LAM-ELISA assay and the Determine TB-LAM-strip assay) in children with suspected tuberculosis (TB) in a high TB/HIV-prevalence setting. They found the assays' sensitivity was higher in HIV-positive versus HIV-negative children: 70% versus 13% for MTB-LAM-ELISA and 50% versus 0% for Determine TB-LAM. In 35 children with excluded active TB, both assays showed a specificity of 97.1%. In addition, LAM excretion declined to zero during or at conclusion of antituberculous treatment in most patients, suggesting its potential as a treatment-monitoring tool [39]. Another recent study found that the urinary LAM level was higher in children with TB compared to non-TB group (p<0.001). Urine LAM had 83% sensitivity and 85% specificity with cut off value 0.98 mg/l using microbiological and clinical confirmation as standard reference. Because the clinical presentation is not specific, the chest X-ray interpretation has low accuracy and sputum sample is difficult to obtain in children, urine LAM test may be a rapid non-invasive alternative for paediatric TB diagnosis [40].

3. Treatment of Tuberculosis

3.1. Chemotherapy of drug-susceptible Tuberculosis

3.1.1. The prestreptomycin era

Between 1925 and 1935, Hart indicated that sanocrysin, a gold salt, was widely used in tuberculosis treatment [41]. A number of different sulphones that had activity in experimental animals were also investigated but were never widely used in treatment. Vitamin D was also explored in early work, as was nicotinamide, from which several current antituberculosis drugs, including isoniazid (INH) and ethionamide, were subsequently developed as analogs. The basis of treatment was, however, rest for the patient in sanatorium and rest for the affected portion of the lung by collapse therapy through operative procedures on the chest wall (thoracoplasty) and the injection of air into the pleural cavity (artificial pneumothorax) [42]. Pulmonary tuberculosis was reputed to have a 50% mortality, with tuberculous meningitis and miliary tuberculosis uniformly fatal.

3.1.2. Early clinical trials on Tuberculosis

After the discovery of streptomycin (SM) [43] and the proof of its antituberculosis activity in the guinea pig, small uncontrolled studies were undertaken in the United States, but the first clinical trial with a randomized intake in the history of medicine was started in1946 by the British Medical Research Council [44]. Because only a limited amount of SM was available in the United Kingdom, patients with advanced pulmonary disease could ethically be randomized to treatment with bed rest alone or bed rest plus 2 g SM daily. The results showed a substantial immediate advantage to the SM arm, but most patients developed SM-resistant strains, and the results of a 5-year follow-up indicated that they had little eventual benefit compared with the control arm. This study focused the aim of development during the next 20 years on preventing the emergence of drug resistance. In contrast to the results in pulmonary tuberculosis, a parallel study showed that SM was able to cure about 44% of patients with tuberculous meningitis [45]. Drug resistance did not emerge in these patients because the bacterial population was too small to contain resistant mutants.

The next step was to conduct a randomized controlled trial (RCT) comparing treatment of acute pulmonary tuberculosis with either SM or p-aminosalicylic acid (PAS) or with both SM and PAS [46]. The aim was to inhibit SM-resistant mutant bacilli with PAS, a very weak drug on its own. The results of this study showed that SM and PAS induced far fewer SM-resistant strains than SM alone. Isoniazid (INH), introduced in 1952, was a more potent drug than SM or PAS, probably because it can be given safely at a dose size substantially above the minimal effective dose. Between 1952 and the mid 1960s, a series of RCTs on combinations of INH, SM and PAS were performed by the British Medical Research Council, the U.S. Veterans Administration, the U.S. Public Health Service, and elsewhere. In 1955, the British Medical Research Council performed the first national drug resistance survey, which showed that almost all strains with primary resistance were resistant to only one drug [47,48]. Because of this finding, treatment with an initial three-drug phase lasting 2 to 3 months, followed by a continuation phase with two drugs was explored first in Scotland and then internationally [49]. Whereas the regimen was highly successful and was adopted as standard in the Western world, it had to be given for at least 12 months with resulting frequent failures to complete treatment. Furthermore, it was too expensive in drug costs (particularly for PAS) to be widely used in developing countries. The need to reduce costs in developing countries led to a series of RCTs in East Africa on thiacetazone (TB1) as a cheaper alternative to PAS. The possible use of INH alone was also explored [50] on drug cost grounds and because the low guinea pig virulence of highly resistant strains suggested that they might not cause progressive human disease [48, 51]. Unfortunately, this thesis proved not to be true [52], and because initial INH resistance carried a poor prognosis, the use of INH in monotherapy has been abandoned.

During the course of these and related studies, it was possible to assess the relative merits of other antituberculosis drugs by their ability to prevent the emergence of INH resistance when used in a double-drug regimen with INH. Whereas rifampin (RMP) was the most effective, SM and ethambutol were a little less effective, and PAS and TB1 much less effective. Estimation of SM and INH concentrations, and later also RMP were made in several different types of tuberculous tissue obtained at resection soon after a drug dose was given. They showed that these drugs penetrated throughout lesions and caseous matter in concentrations adequate for bacteriostatic. Thus, there were no compartments, such as thick-walled cavities, into which these drugs failed to penetrate. Indeed, if this were not true, it would be difficult for combined therapy to be effective because there would always be compartments in which only one drug would be active and would therefore create resistant strains.

From early on in the development of chemotherapy, the rationale for the slow fall in counts of viable bacilli during treatment presented a problem. The existence of persisting bacilli was recognized early and thought likely to occur when bacilli were in a stationary phase of growth or under anaerobic conditions [53,54]. However, perhaps the most important advance in the chemotherapy of tuberculosis was the series of long-term studies of experimental tuberculosis in mice performed in Walsh McDermott's Department at Cornell University on pyrazinamide (PZA) [55]. A model system was established in which treatment with drugs was given to infected mice for periods of 3 months or even longer, and counts of viable bacilli in the organs were done throughout treatment. With slight modifications, this model is still being used today. PZA, reviewed recently [56], was discovered in 1952. It is a remarkable drug that does not appear to have a genetic site of action but accumulates within the bacterial cell, where it acidifies its content and damages membranes. Unlike any other drug, as bacterial metabolism slows down PZA becomes more bactericidal.

In the murine model, therapy with standard drugs-INH, SM, PAS-produced an initial fall in viable counts, but these then leveled out and it was difficult to sterilize the organs. When PZA was added, the counts continued downwards and eventually a state was reached in which all organ cultures were negative. PZA is thus a good sterilizing drug. However, because bacilli in a nonculturable form were still present, relapses eventually occurred. Similar experiments were performed later at the Pasteur Institute, Paris, showing the high sterilizing activity of RMP. In vitro experiments showed that the reason for the high sterilizing activity of RMP probably lay in the speed with which it started to kill bacilli as they recovered from dormancy and not to a particularly rapid kill of slowly growing bacilli. A hypothesis was put forward to explain the activities of different drugs, on the basis of the presence of widely different growth rates within the bacterial population at the start of treatment.

3.1.3. Development of short-course chemotherapy

As a direct result of the two sets of experiments on the treatment of murine tuberculosis with PZA and RMP, the multicenter RCT that established short-course chemotherapy was performed in East Africa in 1970. All of the patients were in hospital throughout treatment to be sure that their prescribed treatment was actually taken. They were allocated at random 6-month regimens of (1) daily SM and INH (SH); (2) SH with the addition of RMP; (3) SH with the addition of PZA; and (4) SH with the addition of TB1, given for 12 months as a control. After completion of treatment the patients were followed up with monthly bacteriology for 24 months. The primary endpoint was the rate of relapse during follow-up and the secondary endpoint was the proportion of patients who had a positive sputum culture at 8 weeks. This was the first RCT in which these end-points were used, which are the same as those currently in operation for modern RCTs. The results show the great reduction in relapse rates in the regimens containing RMP and PZA with a slight superiority of the regimen with RMP. These are the results that led to a burst of RCTs under the auspices of British Medical Research Council in East Africa, Hong Kong, Singapore, Madras, Algeria, and Prague [57]. A few years later they were followed by the licensing of RMP in the United States and subsequent RCTs under the auspices of the U.S. Public Health Service.

When either RMP or PZA was added to a regimen, there was a decrease in the proportion of patients with a positive 2-month sputum culture and a decrease in the relapse rate after treatment, indicating improved sterilizing activity. Furthermore, the addition of RMP to a regimen containing PZA or PZA to a regimen containing RMP also increased sterilizing activity and demonstrated the synergistic sterilizing activity of these two drugs. One regimen emerged from the numerous RCTs. It was a 6-month regimen in which RMP is given throughout, starting with 2 months of SM, INH, RMP, and PZA and is followed by 4 months of INH and RMP (2SHRZ/4HR). This regimen was pioneered in Singapore and its efficacy and low toxicity confirmed by later studies in other countries. It is now widely used with ethambutol substituted for SM (2EHRZ/4RH).

3.2. Chemotherapy of drug-resistant Tuberculosis

Most of drug-resistant Tuberculosis could be administered a standardized therapy (**Table 1**) [58,59]. However, except newly diagnosed multidrug-resistant Tuberculosis (MDR-TB), the treatment of MDR-TB is based on expert opinion and requires the creation of combination drug regimens chosen from five hierarchical groups of first-line and second-line drugs. Such therapy is associated with a high risk of intolerance and serious toxic effects. Regimens may be chosen on a standardized or empirical basis and then switched to individualized therapy after data regarding drug-susceptibility testing become available. However, reliable drug-susceptibility testing is not widely available in regions in which tuberculosis is endemic, particularly for second-line drugs. WHO treatment guidelines for multidrug-resistant tuberculosis recommend that the intensive phase of therapy be administered for at least 8 months.

A fluoroquinolone and an injectable agent should routinely be included to provide a regimen with at least four second-line drugs that will have certain or nearly certain effectiveness, as well as pyrazinamide. Such therapy should be administered for at least 20 months in patients who have not received previous treatment for multidrug-resistant tuberculosis and for up to 30 months in those who have received previous treatment.

An observational study showed that a shorter regimen, with treatment given for 9 to 12 months (the so-called Bangladesh regimen), had acceptable efficacy with fewer adverse reactions in a population with no previous exposure to second-line drugs. In 2016, WHO recommended 9-12 months MDR-TB treatment regimen under specific conditions. To be noticed, it's a conditional recommendation with very low certainty in the evidence. Now this regimen is being more widely evaluated in the ongoing Standardized Treatment Regimen of Antituberculosis Drugs for Patients with Multidrug-Resistant Tuberculosis (STREAM) trial. Since most of the recommended drugs have serious side effects that render treatment particularly difficult, expert consultation is always advised for the treatment of multidrug-resistant tuberculosis.

Extensively drug-resistant tuberculosis is extremely difficult to diagnose and treat in countries in which the disease is endemic. The condition has been associated with death rates as high as 98% among HIV-infected persons.

3.2.1. Category of Antituberculosis drug (Table 2)

3.2.1.1. Fluoroquinolones

Both levofloxacin and moxifloxacin are commonly used to treat MDR-TB. Levofloxacin is more widely available than moxifloxacin, which is more expensive although a reduction in its price is expected in the coming years.

Gatifloxacin is an affordable drug and had been commonly used by TB treatment programmes until the concerns about its dysglycaemic effects led to a global shortage in this medicine. If manufacture of quality-assured formulations of the drug restarts, it could substantially lower the costs of regimens by substituting more expensive options in fluoroquinolones.

Moxifloxacin is relatively easy to administer to older children. However, the tablet must be split to accommodate dosing in younger children and it is highly unpalatable once split or crushed. Levofloxacin is available as a suspension.

3.2.1.2. Second-line injectable agents

These agents present problems to administer intramuscularly or intravenously on a daily basis for several months, often necessitating hospitalization. Giving injections to children and underweight adults is particularly unpleasant and unwelcome.

3.2.1.3. Other agents

Ethionamide and prothionamide are inexpensive, readily available world-wide and easily administered.

Cycloserine has been one of the standard drugs for the treatment of MDR-TB for several years and therefore experience in its use is widespread. Terizidone is less widely used but is available on the GDF Products List.

Clofazimine can be difficult to procure. The implementation of these guidelines at national level needs to ensure that sufficient quantities of this medicine are available to meet the demand and that no stock-outs occur. Moreover, given that there are no good paediatric formulations the capsule contents need to be expressed manually and divided into smaller doses, with risks of incorrect dosing in children.

When linezolid is used, there needs to be close monitoring for side effects, particularly anaemia, thrombocytopenia, lactic acidosis, peripheral neuropathy and optic neuropathy, as these can be severe and life threatening. Historically linezolid has been very expensive, however, it has recently come off patient and the availability of generic products has reduced its market price substantially and it may even decrease further.

3.2.1.4. Add-on agents

Pyrazinamide is inexpensive, readily available and easy to administer.

Isoniazid is inexpensive. It is important to consider the epidemiology of high level versus low level isoniazid mutations in a population before standard treatment regimens including high-dose isoniazid are recommended.

Bedaquiline is a diarylquinoline that blocks ATPase synthesis. There has been no cross-resistance with other drugs so far. Bedaquiline has high tissue binding, which partly accounts for its half-life of more than 24 hours. The early bacterial activity is similar to isoniazid and rifampin after 5 days. For extensively drug-resistant tuberculosis, bedaquiline added to the best available baseline regimen (kanamycin,

Ofloxacin, ethionamide, pyrazinamide, and cycloserine) showed improved clearance. However, despite better bacterial clearance, the bedaquiline treatment group had more deaths than the placebo group in a comparative study. The cause of death did not fit a pattern and may be unrelated to the medication.

Delamanid, a nitro-dihydroimidazoxazole also derived from metronidazole, is effective

in replicating and nonreplicating organisms. Its MIC of 0.006–0.024 mg/ml is impressive; its intracellular activity at 0.1 mg/ml overshadows rifampin at 1–3 mg/ml. It also has delayed killing kinetics. Delamanid plus pyrazinamide and rifampin was superior to rifampin, pyrazinamide, isoniazid, and ethambutol in culture conversion in mice. In humans, delamanid is well tolerated and has good early bacterial activity.

Ethambutol is inexpensive and readily available.

PAS is available through the Global Drug Facility (GDF). Otherwise it is relatively inexpensive and easy to administer.

Amoxacillin-clavulanate is inexpensive and easily obtainable. However, the carbapenems are expensive and are difficult to administer as they must be given two or three times per day via an intravenous line.

Thioacetezone is inexpensive but it has limited availability and it is not currently available through the GDF.

3.2.2. Designing and administrating a MDR regimen

It applies to standardized and individualized regimens. The following are the basic principles involved in the treatment of MDR-TB. Early MDR-TB detection and the prompt initiation of an effective treatment are important factors in obtaining successful outcomes. The intensive phase of MDR-TB treatment should consist of at least four second-line anti-TB drugs that are likely to be effective (including an injectable anti-TB drug), as well as pyrazinamide. Where there is unclear evidence about the effectiveness of a certain drug, this drug can still be part of the regimen, however, it should not be depended upon for success. MDR regimens should include at least pyrazinamide, a fluoroquinolone, an injectable anti-TB drug, ethionamide (or prothionamide) and cycloserine. The drugs in the regimen should be judged to be "likely effective" [58]. An anti-TB drug is considered "likely to be effective" when:

– The drug has not been used in a regimen that failed to cure the individual patient;

– DST performed on the patient's strain indicates that it is susceptible to the drug (DST for isoniazid, rifampicin, Groups 2 and 3 drugs is considered reliable; DST for all other drugs is considered not reliable enough for individual patient management);

– No known resistance to drugs with high cross-resistance;

– No known close contacts with resistance to the drug;

– Drug resistance surveys demonstrate that resistance is rare to the drug in patients with similar TB history. This final criterion is relevant in the absence of DST or for drugs in which in-

dividual DST is not reliable. Note: It is not always possible that information of all five criteria can be ascertained. Therefore, clinical judgment is often necessary on whether to count a drug as "likely effective".

There are conditions when more than five drugs are used. These conditions would be applicable when the effectiveness for a drug(s) is unlikely or questionable. One such relatively common condition is the treatment of XDR-TB.

3.2.3. Treatment strategies for MDR-TB and XDR-TB

Drugs that the patient is known to have a strong contraindication of usage due to drug–drug interactions, overlying toxicities, co-morbidities, history of severe allergy or other adverse reactions, and/or pregnancy-should not be used. Every drug should be administered with a right dosage (**Table 3**). A fluoroquinolone should be used (strong recommendation, very low quality evidence). A later-generation fluoroquinolone rather than an earlier-generation fluoroquinolone should be used (conditional recommendation, very low quality evidence). In the treatment of patients with MDR-TB, ethionamide (or prothionamide) should be used (strong recommendation, very low quality evidence). This recommendation assumes the recommended drugs meet the criteria of "likely to be effective" and there are no contraindications to its use (such as severe adverse effects). The intensive phase (i.e. the initial part of treatment during which a Group 2 injectable agent is used) lasts at least eight months in total, but the duration can be modified according to the patient's response to treatment. The optimal duration of intensive phase following culture conversion, which is associated with treatment success, could not be inferred directly from the analysis used to revise the WHO programmatic management of drug-resistant TB guidelines in 2011.

Some clinical experts may prefer that the intensive phase is continued for at least four months past culture conversion. The total length of treatment is expected to be at least 20 months in most patients not previously treated for MDR-TB. Some clinical experts may prefer that total treatment be for at least 12 months past the point at which culture converts to negative and, some others may prefer not to give less than 20 months in total. Each dose is given under a patient-centered directly observed therapy (DOT) throughout the treatment. A treatment card is marked for each observed dose. DOT can be performed either at facility-based or community-based levels, keeping in mind that social support is an essential component of care and treatment delivery. Any adverse effects of drugs should be managed immediately and adequately to relief suffering, minimize the risk of treatment interruptions, and prevent morbidity and mortality due to serious adverse effects. Antiretroviral therapy (ART) is recommended for all patients with HIV and drug-resistant TB, irrespective of CD4 cell-count, as early as possible (within the first eight weeks) following initiation of the anti-TB treatment (strong recommendation). The drug dosage is usually determined by age and weight. Pyrazinamide,

ethambutol and fluoroquinolones should be given once a day. Depending on patient tolerance, once-a-day dosing is also used for oral second-line anti-TB drugs from Group 4, however, ethionamide/prothionamide and cycloserine have traditionally been given in split doses during the day to reduce adverse effects. All anti-TB drugs can be started at full dose. However, if tolerance is an issue, cycloserine, ethionamide and PAS dosing can be increased gradually over a two-week period. Injectable drugs can be given five to seven days a week depending on the availability of a skilled medical person to give the intramuscular injections. Injectable anti-TB drugs should be given once daily, i.e. do not split the dose over the day. If adverse effects are problematic in a patient, the injectable agent may be given three times a week, preferably only after culture conversion. When possible oral drugs are to be given seven days a week under directly observation. Some programmes suggest giving all drugs six days a week, but it is not known if this is equal to seven days a week. Oral drugs should not be given five days a week (only the injectable agent is allowed to be on a five days a week schedule, see above). Pyrazinamide can be used for the entire treatment. Many drug-resistant TB patients have chronically inflamed lungs, which theoretically produce the acidic environment in which pyrazinamide is more effective. Alternatively, in patients doing well, pyrazinamide can be stopped with the injectable drug if the patient can continue with at least three likely effective drugs. In MDR treatment strategies that initially enrolled patients based on their strain being resistant to rifampicin alone, isoniazid may be included in the MDR regimen until DST to isoniazid can be done to determine if the isoniazid should be continued. Patients with MDR-TB should be treated using mainly ambulatory care rather than models of care based principally on hospitalization.

3.2.4. Outcome of MDR-TB

Definitions of treatment outcomes for drug-resistant patients are as following [58]:

Cured: Treatment completed as recommended by the national policy without evidence of failure AND three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase.

Treatment completed: Treatment completed as recommended by the national policy without evidence of failure BUT no record that three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase.

Treatment failed: Treatment terminated or need for permanent regimen change of at least two anti-TB drugs because of: Lack of conversion by the end of the intensive phase; or Bacteriological reversion in the continuation phase after conversion to negative; or Evidence of additional acquired resistance to fluoroquinolones or second-line injectable drugs; or Adverse drug reactions.

Died: A patient who dies for any reason during the course of treatment.

Lost to follow-up: A patient whose treatment was interrupted for two consecutive months or more.

Not evaluated: A patient for whom no treatment outcome is assigned. (This includes cases "transferred out" to another treatment unit and whose treatment outcome is unknown).

Treatment success: The sum of Cured and Treatment completed.

For Treatment failed, lack of conversion by the end of the intensive phase implies that the patient does not convert within the maximum duration of the intensive phase applied by the programme. If no maximum duration is defined, an 8-month cut-off is proposed. For regimens without a clear distinction between intensive and continuation phases, a cut-off eight months after the start of treatment is suggested to determine when the criteria for Cured, Treatment completed and Treatment failed start to apply.

The terms "conversion" and "reversion" of culture as used here are defined as follows: Conversion (to negative): culture is considered to have converted to negative when three consecutive cultures, taken at least 30 days apart, are found to be negative. In such a case, the specimen collection date of the first negative culture is used as the date of conversion. Reversion (to positive): culture is considered to have reverted to positive when, after an initial conversion, two consecutive cultures, taken at least 30 days apart, are found to be positive. For the purpose of defining Treatment failure, reversion is considered only when it occurs in the continuation phase.

Table 1: Standardized regiment for drug-susceptible and drug-resistant tuberculosis

Category	Recommended Regimen	Lowest duration
Drug-susceptible[a]	2HRZE/4HR	6 months
Resistant to H(±S)	RZE±FQ[b]	6-9 months
Resistant to H+E(±S)	RZFQ	9-12 months
Resistant to H+E+Z(±S)	RFQPto+SLIA 2-3 months	18 months
Resistant to R	MDR-TB regimen +H	20 months
MDR-TB[c]	8ZAk(Cm)Mfx(Lfx)Pto(CS)Cfz(CS)/12 ZMfx(Lfx)Pto(CS)Cfz (CS)	20 months

Abbreviations:
[a] For extrapulmonary tuberculosis, treatment should last at least 1 year.
[b]Fluoroquinolone
[c]Newly-diagnosed MDR-TB

Table 2

Drug	Weight(kg)			Maximam dosage	Frequency
	<33a	<50	≥50		
Isoniazid (H)	10mg/kg	300mg	300mg	300 mg	qd
Rifampin (R)	10-20mg/kg	450mg	600mg	600mg	qd
Ethambutol (E)	15-25mg/kg	7500mg	1000mg	1500mg	qd
Pyrazinamide (Z)	30-40mg/kg	1500mg	1750mg	2000mg	qd or tid
Rifabutin (Rfb)	-b	150-300 mg	300mg	300mg	qd
Rifapentine(Rpt)	10-20mg/kg	450mg	600mg	600mg	biw
Streptomycin (S)	15-20mg/kg	750mg	750mg	1000mg	qd
Amikacin (Ak)	15mg/kg	400mg	600mg	800mg	qd
Capremycin (Cm)	15-20mg/kg	750mg	750mg	1000mg	qd
Levofloxacin (Lfx)	10mg/kg	400mg	500-600mg	750mg	qd
Moxifloxacin (Mfx)	7.5-10mg/kg	400mg	400mg	400mg	qd
Gatifloxacin (Gfx)	-	400mg	400mg	400mg	qd
Prothionamide (Pto)	15--20mg/kg	600mg	800mg	800mg	bid or tid
Cycloserine (Cs)	15-20mg/kg	500mg	750mg	1000mg	bid or tid
p-aminosalicylic acid (P)	200-300mg/kg	8g	10g	12g	qd
Linezolid (Lzd)	-	600mg	600mg	1200mg	qd
Clofazimine (Cfz)	-	100mg	150mg	300mg	qd
Amocixilline/Cla-vulate (Amx/Clv)	50 mg/kg	1300mg	1950mg	-	bid or tid
Clarithromycin (Clr)	7.5mg/kg	500	750	1000	bid or tid
Imipenem (Imp)	60mg/kg	1500	2000	2000	bid or tid

Abbreviations:
[a]for children and adults whose weight are less than 33 kg.
[b]no data
Dosage and frequency of antituberculosis drug. All patients should be administered with drugs based on their weight.

Table 3: Medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant TB[1]

A. Fluoroquinolones[2]	Levofloxacin	Lfx
	Moxifloxacin	Mfx
	Gatifloxacin	Gfx
B. Second-line injectable agents	Amikacin	Am
	Capreomycin	Cm
	Kanamycin	Km
	(Streptomycin)[3]	(S)

C. Other core second-line agents[2]	Ethionamide / Prothion-amide		Eto / Pto
	Cycloserine / Terizidone		Cs / Trd
	Linezolid		Lzd
	Clofazimine		Cfz
D. Add-on agents (not part of the core MDR-TB regimen)	D1	Pyrazinamide	Z
		Ethambutol	E
		High-dose iso-niazid	Hh
	D2	Bedaquiline	Bdq
		Delamanid	Dlm
	D3	p-aminosalicylic acid	PAS
		Imipenem-cilas-tatin[4]	Ipm
		Meropenem[4]	Mpm
		Amoxicillin-clavulanate[4]	Amx-Clv
		(Thioacetazone)[5]	(T)

1. This regrouping is intended to guide the design of conventional regimens; for shorter regimens lasting 9-12 months the composition is usually standardized

2. Medicines in Groups A and C are shown by decreasing order of usual preference for use

3. Resistance to streptomycin alone does not qualify for the definition of extensively drug-resistant TB (XDR-TB)

4. Carbapenems and clavulanate are meant to be used together; clavulanate is only available in formulations combined with amoxicillin

5. HIV-status must be tested and confirmed to be negative before thioacetazone is started

3.3. Treatment of extrapulmonary Tuberculosis

In most cases of extrapulmonary tuberculosis there are many fewer organisms present. In general, regimens used for pulmonary tuberculosis are effective in the treatment of extra-pulmonary tuberculosis. WHO recommends classification of the disease into severe and non-severe forms. Severe forms include meningeal and central-nervous-system tuberculosis, spinal tuberculosis, abdominal tuberculosis, bilateral pleural effusion, pericardial effusion, and bone and joint tuberculosis involving more than one site. All major organisations agree that some forms of disease, such as meningitis, may benefit from a longer treatment course. Steroids

should be used for patients with meningitis, particularly with neurological impairment, since these drugs are likely to decrease morbidity and mortality in such cases. A RCT performed in Vietman show that adjunctive treatment with dexamethasone improves survival in patients over 14 years of age with tuberculous meningitis but probably does not prevent severe disability [60]. However, another RCT show in patients with tuberculous pericarditis, neither prednisolone nor M. indicus pranii had a significant effect on the composite of death, cardiac tamponade requiring pericardiocentesis, or constrictive pericarditis [61].

3.4. Surgery for Tuberculosis

Surgery has been employed in treating TB patients since before the advent of chemotherapy. In many countries it remains one of the treatment options for TB. With the challenging prospect in many settings of inadequate regimens to treat multidrug and extensively drug-resistant TB, and the risk for serious sequelae, the role of pulmonary surgery is being re-evaluated as a means to reduce the amount of lung tissue with intractable pathology, to reduce bacterial load and thus improve prognosis.

Indications for surgery include: persistently positive smear or sputum culture for acid-fast bacilli despite aggressive chemotherapy; high risk of relapse (based on drug resistance profile and radiological findings); complications of tuberculosis including bronchiectasis, empyema, haemoptysis; sufficient drug treatment available (to reduce bacterial burden and allow healing of bronchial stump) [58].

Preoperative work-up include: chest CT scan to assess extent of disease and guide surgical resection; pulmonary function testing; and ventilation perfusion scan to ensure presence of adequate pulmonary reserve to tolerate surgery; bronchoscopy to rule out endobronchial tuberculosis, contralateral disease, and malignancy; echocardiogram to rule out heart failure and pulmonary hypertension; nutritional assessment to ensure patient can tolerate and recover from surgery.

4. Future Perspectives

Although a great deal of progress have been achieved in the past decades. However, no new first-line drugs have been discovered for several decades. The influence of HIV infection on the tuberculosis burden and MDR-TB will be difficult to reverse. Further progress will require continued rigorous and dedicated application of current technology and will be greatly facilitated by the discovery and widespread application of new diagnostic techniques, drugs, and prevention strategies, such as an effective vaccine. There is still a long road to eliminate TB. The future diagnosis should be some high accuracy methods with less turnout time, and the future treatment should be short and less interactive with other drugs (such as anti-retrovirus agent) and effective vaccine.

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