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REVIEW PAPER

Tuberculosis challenges: Resistance, co-infection, diagnosis, and treatment

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ABSTRACT

Early diagnosis of tuberculosis (TB), followed by effective treatment, is the cornerstone of global TB control efforts. An estimated 3 million cases of TB remain undetected each year. Early detection and effective management of TB can prevent severe disease and reduce mortality and transmission. Intrinsic and acquired drug resistance of *Mycobacterium tuberculosis* (MTB) severely restricted the anti-TB therapeutic options, and public health policies are required to preserve the new medications to treat TB. In addition, TB and HIV frequently accelerate the progression of each other, and one disease can enhance the other effect. Overall, TB-HIV co-infections show an adverse bidirectional interaction. For HIV-infected patients, the risk of developing TB disease is approximately 22 times higher than for persons with a protective immune response. Analysis of the current TB challenges is critical to meet the goals of the end TB strategy and can go a long way in eradicating the disease. It provides opportunities for global TB control and demonstrates the efforts required to accelerate eliminating TB. This review will discuss the main challenges of the TB era, including resistance, co-infection, diagnosis, and treatment.

KEYWORDS

tuberculosis, Mycobacterium tuberculosis, coinfection, diagnosis, treatment, resistance

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INTRODUCTION

Tuberculosis (TB) is an ancient disease that remains a significant public health problem worldwide. Based on the World Health Organization (WHO) reports, an estimated 10 million persons fall ill with TB annually [1]. A better understanding of the current challenges of TB is one of the critical elements for a faster decrease in TB prevalence to reach the targets

of end TB strategy [2]. Despite recent advances in the diagnosis and treatment of TB, several challenges continue to slow progress towards eradicating the disease. The new TB diagnostics methods remain a challenge, especially in the high burden regions. Rapid diagnosis of *Mycobacterium tuberculosis* (MTB), especially in high burden countries, is the main problem. Although recently several assays were developed to diagnose TB, none of these methods are yet completely satisfactory tools [3].

Although TB control has been effective in some world regions, these gains are threatened by the increasing burden of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB. XDR-TB has evolved in several tuberculosis-endemic countries to drug-incurable or programmatically incurable tuberculosis (totally drug-resistant tuberculosis). This poses several challenges similar to those encountered in the pre-chemotherapy era, including the inability to cure tuberculosis, high mortality, and the need for alternative methods to prevent disease transmission [4].

MTB co-infection in cases pre-infected with HIV and/or with full-blown acquired immune deficiency syndrome (AIDS) is the significant threat of emergence of a pandemic. Because of raised respect for the morbidity and mortality related to this problem, the WHO suggests aggressive strategies for MTB diagnosis in first visits related to HIV screening and treatment [5, 6]. Although this condition of co-infection is a significant global health concern in resource-constrained settings with a high burden of both diseases, such as Africans and Asian peoples, it is being gradually documented in the settings of industrialized countries. WHO expected 1.5 million tuberculosis (TB) fatalities in about 70% HIV-negative and 30% HIV-positive cases, making MTB the second important cause of mortality from an infectious disease and the prominent cause of mortality in the setting of AIDS around the world [7].

Although tuberculosis challenges have been partially reported in several types of research worldwide, there has been no comprehensive published data on these challenges. Therefore, we performed this systematic review to update data related to co-infections with TB, diagnosis, the introduction of new drugs and new regimens for treatment, and mechanisms of resistance and multi-drug resistance, among TB isolates.

METHODS

Search strategy

We performed a comprehensive literature search based on keywords (Antibiotics, Antimicrobial, *M. tuberculosis*, Mechanisms, polymerase Chain Reaction (PCR), Multidrug-resistant Tuberculosis (MDR-TB)). The main focus of this review was resistance, TB/HIV and TB/HBV co-infections, drugs available for the treatment of MDR-TB, and diagnosis. In the present review, a search was restricted to original English articles.

Inclusion and exclusion criteria

Present review included peer-reviewed papers presenting cross-sectional studies. The reference lists of included studies and previous reviews will be explored to identify other eligible studies. We excluded studies of *Mycobacterium* other than tuberculosis, duplicate articles, bulletins, editorials, abstracts reported in conferences, grey literature, and letters to the editor.

ANTIBIOTIC RESISTANCE

Intrinsic resistance

Fosfomycin. Cell wall permeability of MTB is an effective barrier for drug penetration. The mycobacterial cell wall is extraordinarily thick, and the mycolic acid structure determines the fluidity of the cell wall [8]. MTB is intrinsically resistant to fosfomycin (FOS), as its corresponding cysteine residue is changed into aspartic acid (Table 1). Through covalent modification of a highly conserved cysteine residue in the active site of uridine diphosphate N-acetylglucosamine enolpyruvyl transferase (MurA), FOS inactivates MurA. Since MTB encodes an aspartic acid residue instead of cysteine in the active site of MurA, it is naturally FOS resistant [9].

Carbapenems. Carbapenems target transpeptidases producing the unusual transpeptide linkages, and approximately 80% of these sorts of linkages are found in the MTB peptidoglycan layer. LdtMt2, transpeptidase type 2, is the main transpeptidase among MTB isolates and is critical for virulence, cell wall synthesis, and amoxicillin (AMX) tolerance of MTB. Meropenem (MER) clavulanate inactivates β-lactamase and prevents the formation of transpeptide linkages. Therefore, the inactivation of LdtMt2 is the main mechanism of MER-clavulanate to kill *MTB* effectively [10].

Penicillins. The class A penicillin-binding protein PonA2 plays a complex role in MTB physiology and is spotted as a promising target for inhibitors. PonA2 is involved in the adaptation of MTB to dormancy, an ability that has been attributed to the presence of a C-terminal PASTA module in its sequence. Analysis of the binding properties of the PASTA domain from PonA2 shows that it cannot bind to muropeptides, β-lactams, or polymeric peptidoglycan [11]. Ag85, the antigen 85 complex from MTB, consists of FbpA, FbpB and FbpC2 secreted proteins, which play an essential role in TB pathogenesis [12].

Porins and efflux pumps. Mycobacterial porins import the nutrients and small molecules required for the growth of MTB. They can also import the drugs through the outer layer of the cell wall. Trans-expression of *Mycobacterium smegmatis* porin A (MspA) increases the susceptibility of *Mycobacterium* bovis and MTB to several antibiotics such as isoniazid (INH), streptomycin (STR), ethambutol (ETB),

Table 1. Mechanisms of Resistance in *Mycobacterium tuberculosis*

Intrinsic Resistance			Acquired Resistance	
Mechanisms		Antibiotics	Mechanisms	Antibiotics
Cell wall Impermeability	Corresponding cysteine residue is changed into aspartic acid	Fosfomycin	inhA, katG, ndh ahpC, kasA	Isoniazid
			rpoB	Rifampicin
	L,D transpeptidases	β-lactam	pncA, rspA, panD	Pyrazinamide
Enzymatic modification of antibiotics	Acetyltransferase, Phosphotransferase	Aminoglycosides	embCAB, embR	Ethambutol
			mmpL3	SQ109
	RIF ADP-Ribosyltransferase	Rifampicin (In *M. smegmatis*)	Mutations	
			rpsL, rrs, gidB	Streptomycin
Modification of Targets	erm	Macrolides	whiB7	
	RbpA	Rifampicin	Rrs, eis, whiB7	Amikacin/ kanamycin
	mfpA	Quinolones		
	MethyltransferasetlyA is deactivated	Capreomycin, Viomycin	rv0636	NAS-21 and NAS-91
Enzymatic degradation of antibiotics	β-lactamase, low cell envelope permeability and presence of low protein binding affinity for beta-lactams	β-lactams	rrl	Macrolide-ketolide
			blaC, ponA, Pbp	B-Lactams
Porin channels	Deletion of the MspA	Resistance of *M. smegmatis* to several agents	ethA, ethR inhA, ndh mshA	Ethionamide
			nfnB, dprE1	Benzothiazinones
			rrl, rplC	Oxazolidinones
			gyrA, gyrB	Fluoroquinolones
			rv0678, rv2535c, rv1979c	Clofazimine
Efflux pumps	Increased transcription of jefA	Isoniazid, Ethambutol, Streptomycin	atpE	Bedaquiline
	Overexpressed drrA, drrB, efpA, mmr, and RV1217-Rv1218 efflux pumps	Isoniazid, Rifampicin	alrA, cycA, ddl, Ald	D-Cycloserine
			thyA, dfrA, folC, ribD	P-Aminosalicylic acid
			16S Rrna 16S rRNA	Tetracyclines and glycylcyclines
Dormancy and Latency	Dormant state	Isoniazid and rifampicin	dfrA, sulI, folP1	Trimethoprim and sulfonamides
Activation of Transcriptional Regulator	whiB7	Several antibiotics	tlyA, rrs	Capreomycin
	DosR	One of the key regulators that mediate MTB survival within granulomatous lesions		
	SigF	Intrinsic MDR phenotypes		

and β-lactams. Furthermore, deletion of *M. smegmatis* porin C (MspC) genes leads to increased resistance to other drugs, including rifampicin (RIF), erythromycin (ERY), and vancomycin (VAN) [8].

MTB fights host immunity and chemotherapy on various levels, such as repairing or degrading oxidized proteins that are not reversible (IOPs). New findings have reported that MTB applies mechanisms to bear stresses exerted by the host, including being exposed to reactive oxygen species (ROS), reactive nitrogen species (RNS), carbon monoxide, acid, cupric ions, and antimicrobial peptides (AMP), as well as deprivation of oxygen, iron, and nutrients, which all influence multiple pathways [13]. The growth of progeny, which inherited more IOPs, is slower than those that inherit less, and they probably die upon exposure to antibiotics again. It is believed that ClpB is a marker of IOPs that mediates the resistance against proteotoxic stress as well. MTB without ClpB cannot recover following the stationary phase or being exposed to antibiotics *in vitro*; besides, they are less infectious than their wild-type and complemented counterparts [14].

Five super families of efflux systems among MTB isolates include small MDR, major facilitator super family, ATP binding cassette, the multi-drug, toxic compound extrusion, and resistance nodulation division family. When MTB enters the human macrophage, mycobacterial efflux proteins are activated by antibiotic pressures and extrude a wide range of drugs from the cytoplasm to the external environment. Efflux pumps confer both single and multiple drug resistance [15, 16].

The LfrA present in *M. smegmatis* was the first efflux pump featured in mycobacteria; its expression takes place in multicopy plasmids. Other efflux pumps featured in mycobacteria were TetV causing resistance to tetracycline, and Tap, causing low-level resistance to aminoglycosides and tetracycline upon overexpression in *M. smegmatis*. These findings highlight diverse studies on drug efflux in MTB bacterium [17].

One of the efflux proteins is Rv1258c, which is a proteinaceous active transporter. Its gene is responsible for encoding a tetracycline/aminoglycoside resistance (TAP-2)-like efflux pump. It has been shown that the vulnerability of the organism to these two drugs is increased following the deletion of this gene from the *M. bovis* bacillus Calmette-Guérin (BCG) chromosome. This gene is significantly involved in MDR-TB; it has been shown that cytosolic accumulation of drugs is prevented since this tap-like pump's drug resistance and transcription levels are linked. It is believed that Rv1258c is overexpressed under RIF pressure; hence, it could be said that this crucial efflux protein is involved in efflux-mediated RIF resistance [18].

When MTB enters the human macrophage, mycobacterial efflux proteins are activated by antibiotic pressures and extrude a broad range of drugs from the cytoplasm to the external environment *In vitro*, macrophage-induced tolerance is prevented by a calcium channel antagonist named "verapamil." Verapamil as a calcium channel blocker is displayed that can use to treat certain heart rhythm disorders, paroxysmal supraventricular tachycardia, angina (chest pain), atrial fibrillation/flutter, and hypertension. Therefore, efflux pump inhibitors target bacterial growth and drug tolerance [19].

Aminoglycosides. Kanamycin (KAN) and amikacin (AMK) are important bactericidal aminoglycosides used to treat MDR-TB, and resistance to one or both of these drugs is a defining characteristic MDR-TB. The production of aminoglycoside-modifying enzymes (AME) is by far the most common mechanism of aminoglycoside resistance; three classes of these enzymes, namely aminoglycoside acetyltransferase, aminoglycoside phosphotransferase, and aminoglycoside nucleotidyltransferase, have been identified [20]. Zaunbrecher et al. observed mutations in the −10 and −35 promoter region of the *eis* gene responsible for encoding a previously uncharacterized aminoglycoside acetyltransferase in MTB isolates. These mutations increase the amount of *eis* leaderless mRNA transcript 20–180-fold and a corresponding increase in protein expression. Moreover, 80% of clinical isolates have low-level kanamycin resistance to harbored eis promoter mutations [21, 22].

Macrolides and fluorochinolones. Mycobacteria have natural susceptibility to clarithromycin, and they have become clinically resistant due to mutation in the 23S rRNA gene. Nevertheless, studies show that the presence of the *erm* gene results in the main mechanism of clinically significant macrolide resistance acquisition in other pathogens. Thus, it is necessary to pay attention to the new *erm* genes described recently for *M. smegmatis* and MTB that have intrinsic resistance to macrolides. In most bacteria, the expression of an *erm* gene leads to resistance to macrolide, lincosamide, and streptogramin B (MLS) agents. Nevertheless, it seems that the mycobacterial *erm* genes cause resistance limited to ML agents [23].

Exploring macrolide resistance determinants within the genome of MTB has shown the presence of a sequence that encodes a putative rRNA methyltransferase. The deduced protein is comparable to Erm methyltransferases (ErmMT), leading to MLS resistance by methylation of 23S rRNA. Although the parallel gene (*ermMT*) is present in MTB, it is not present in non-tuberculosis mycobacteria (NTM), and NTM isolates outperform MTB in terms of sensitivity [24]. FLQs have been favored recently in TB treatment.

Ciprofloxacin (CIP) and sparfloxacin (SPR) resistance is brought about following the expression of *M. tuberculosis* pentapeptide repeat protein MfpA. MfpA binds to DNA gyrase and impedes its activity. According to the sequence of this protein, it is a member of the pentapeptide repeat family of bacterial proteins, in which every fifth amino acid is either leucine or phenylalanine. MTB involves a 183–amino acid MfpA homolog encoded by the *Rv3361c* gene; in other words, it is 67% like the 192-residue *M. smegmatis* MfpA protein [25].

Capreomycin is a macrocyclic peptide drug produced by *Saccharothrix mutabolis* subspecies *capreolus* to treat MDR-TB. Maus et al. isolated and characterized capreomycin-

resistant MTB and *M. smegmatis* isolates to explore the basis of resistance to this antibiotic. The transposon insertion site of one mutant was plotted to an open reading frame in the unfinished *M. smegmatis* genome corresponding to the *tlyA* gene (Rv1694) in the MTB H37Rv genome [26].

Rifampicin. RIF is currently the main standard chemotherapeutic regimen for active TB that impedes the transcription activity of prokaryotic RNA polymerase. The interaction of RIF with the beta subunit of RNA polymerase has been reported. The discovery of RbpA made this mechanism more diverse.

RbpA is a new RNA polymerase-binding protein in *Streptomyces coelicolor* that is capable of lessening the effect of RIF on RNA polymerase activity. MsRbpA is a homologue of RbpA in *M. smegmatis* [27]. It is believed that intrinsic resistance is caused by the mycobacterial transcriptional regulator whiB7 following the activation of its expression and many antibiotic resistance genes in response to drugs. According to a study by Burian et al., whiB7 was transcribed from a promoter kept across mycobacteria, including an AT-rich sequence probably targeted by WhiB7 [28].

Compounds with varied structures and targets induce expression, which does not rely on the mediating capability of whiB7 to mediate resistance and depend on media composition. It has been shown that the intrinsic antibiotic resistance was increased clinically following pretreatment with whiB7 activators. It was reported that the reductant dithiothreitol increased drug-induced transcription synergistically; this is reflected by a shift that relies on whiB7- to a highly reduced cytoplasm shown by the ratio of decreased/oxidized mycothiol [28].

A regulon of 48 constituent genes called the dormancy survival regulon, named DosR regulon, is encoded by the MTB genome. The transcription factor, DosR, is up-regulated by the inhibition of aerobic respiration; thus, it seems that there is a link between the controls of the regulon and the physiology of respiration in MTB. Hypothetical proteins are encoded by various constituent genes of DosR regulon. However, shedding light on the conditions for up-regulation of these genes would probably reveal their involvement in MTB adaptation to the host environment [29–31].

ACQUIRED RESISTANCE

Isoniazid

It has been shown that some factors are involved in the acquired resistance to commonly used antibiotics in TB treatment. These are linked with natural mutations that lead to the target's overexpression (e.g., for INH/ETM in the promoter region of *inh*A), compromising pro-drug activation. Nevertheless, it is not possible to explain the resistance phenotypes of a considerable proportion of clinical isolates of MTB by these mutations only; roughly 5% of strains resistant to RIF and up to 30% of strains resistant to INH do not undergo mutations in the known resistance genes (Table 1) [32].

Rifampicin

Most RIF-resistant MTB clinical strains harbor *rpoB* gene mutations which code for the β-subunit of the RNA polymerase. Consequently, due to some conformational changes, the affinity for the antibiotic is reduced, and resistance is boosted. In more than 95% of RIF -resistant MTB strains, there are mutations in the "hot-spot region" of 81-bp spanning codons 507–533 of the *rpoB* gene, which is called the RIF resistance-determining region as well [33]. Mutations in codons 516, 526, and 531 are highly linked mutations with resistance to RIF in most of the studies. Few reports revealed the occurrence of mutations outside the hot-spot region of *rpoB*. Cross-resistance with other RIFs is also possible. It is acknowledged that mutations in some codons such as 518 or 529 are linked to low-level RIF resistance, which is still vulnerable to other RIFs (e.g., rifabutin or rifalazil) [34].

Streptomycin

Data on the genetic basis of resistance to several anti-TB agents are scarce. For example, resistance to STR was observed through *rrs* and *rpsL* mutations that alter the STR-binding site; yet, these changes have been identified in just over one-half of the isolates [34]. Consequently, resistance mechanisms should be investigated by conducting more studies. Resistance to INH is a good example. The catalase enzyme activity and high-level INH resistance are eliminated or reduced by point mutations, partial or total deletions, or insertions in the *kat*G gene. The activating INH to the active hydrazine derivative necessitates the activity of the catalase enzyme. High-level resistance is expected due to deficient catalase activity, which is found in roughly 85% of MTB isolates resistant to INH [35].

On the other hand, a point mutation in the regulatory region of the *inhA* operon leads to low-level INH resistance that would overexpress *inhA*. Isolates with this mutation are normal in terms of mycolic acid synthesis; however, they have low-level INH resistance. Point mutations have been shown in the regulatory region of *ahpC* that counter the effects of the absent or decreased catalase function and do not cause resistance directly [36].

A study was performed in Italy to identify INH resistance gene mutation and reported that these genes are *katG*, *ahpC*, *inhA*, and *kasA*. Although *inhA* mutations were more common among INH -resistant MTB isolates, *katG* 315 mutations were more common among MDR MTB isolates. In a study on the INH -resistant MTB strains in China, 82% were positive for *katG* 315 mutation and 18% for *inhA* mutation. Another study performed on INH -resistant MTB strains isolated from South America with a high burden of drug-resistant TB showed *katG*, *ahpC*, and *inhA* mutations. In this survey, *katG* mutations were observed in 81% of isolates, in which 98% was contributed to *katG* S315T mutation [37].

Pyrazinamide

A new target of pyrazinamide (PYR), the ribosomal protein S1 (rpsA), was introduced by Shi et al. They noticed its overexpression that leads to PYR resistance. Additionally, Shi et al. noticed that mutation in the *panD* gene that is responsible for encoding aspartate decarboxylase would probably bring about PYR resistance in isolates with no *pncA* and *rpsA* mutations.

In another study, PYR vulnerability test for 142 MDR-TB clinical isolates from a Chinese state TB referral center and sequenced *pncA*, *rpsA*, and *panD* genes confirmed the link between them and PYR resistance [38, 39].

Ethionamide

Ethionamide (ETM) and INH antibiotics are analog in terms of morphology, which impede mycolic acid's biosynthesis. The existence of partially cross-resistant phenotypes has long been confirmed. Low-level INH-resistant isolates regularly have low-level resistance to ETM, whereas high-level INH -resistant strains usually have susceptibility to ETM. It is believed that these two antibiotics have a common molecular target because they are alike in terms of morphology and the presence of cross-resistant phenotypes [40]. Researchers have shed light on the biochemistry of ETM and the mechanistic relationship of this antibiotic to INH by detecting and characterizing the *ethAR* loci [41]. ETM should be activated through an EthA-mediated process like the *KatG* activation of INH. The putative final metabolites for both antibiotics are alike, and their cellular target is the same (*InhA*) [42]. Genetic alterations decrease EthA activity that expectedly would increase resistance to ETM, just as *katG* mutations lead to resistance to INH [42].

Bedaquiline

The first certified drug to treat MDR-TB is bedaquiline (BDQ), which is an ATP synthase inhibitor. It has been shown that BDQ resistance was because of target-based mutations *in vitro*. Although several target-based resistance mutations in the atpE gene have been shown so far, target-based mutations were observed in only 30% (15/53) of the isolates upon applying a Luria-Delbruck fluctuation assay generating a more extensive set of BDQ-resistant mutants, indicating an extra resistance mechanism. The mutation rates in the above study revealed a genotypic instead of a phenotypic resistance mechanism. However, genomic changes were not observed in whole-genome sequencing of two non-*atpE* mutants initially [43].

Clofazimine

Data on the mechanisms of clofazimine (CLO) resistance in MTB isolates are insufficient. Recent studies have shown that cross-resistance is made between CLO and BDQ *in vitro* due to the mutations in Rv0678, which is responsible for encoding the MarR-like transcriptional regulator of the MmpS5-MmpL5 efflux system. Actually, mutations in Rv0678 are the main mechanism of resistance to CLO among isolates selected with CLO *in vitro*. According to the clinical trials of BDQ, isolates that are less susceptible to BDQ have *Rv0678* mutations and cross-resistance to CLO(44).

Nevertheless, data on the occurrence of strains resistant to CLO and mutations in *Rv0678* in clinical strains of MTB are insufficient. It has been reported that mutations in the putative proline aminopeptidase pepQ (Rv3525c) lead to low-level BDQ and CLO cross-resistance *in vitro* and mice; however, they should be confirmed among clinical isolates [44].

Cycloserine

Cycloserine (CYC) is a broad-spectrum antibiotic regularly applied as a second-line drug to treat MDR-TB, which is capable of blocking both D-alanine racemase (Alr) and D-alanine-D-alanine ligase (Ddl). The overexpression of AlrA is the main mechanism of CYC resistance. In the N-terminal domain of AlrA, pyridoxal phosphate as a cofactor is linked to a lysine within the active site. The bond between pyridoxal phosphate and lysine is broken by CYC, which leads to the formation of an alternative covalent bond with pyridoxal phosphate, thus, leading to the overexpression of AlrA [45].

Multidrug therapy is one of the most powerful strategies for treating tuberculosis (TB) patients and inhibiting drug-resistant mutants during treatment. The use of fixed-dose combinations (FDCs) for the treatment of TB has various advantages, including simple therapy, uncomplicated drug management, and the decreased possibility of monotherapy. It is speculated that by preventing monotherapy, FDCs can restrict the risk of emergence of drug-resistant tuberculosis; however, this claim needs validation [46].

Based on the Medical Research Council of the United Kingdom documentation in 1950, combination therapy utilized for the first time to treat TB was the use of STR and *para*-aminosalicylic acid. These antimicrobial agents were the outcomes of long-term surveys on organism-derived antibiotics and synthetic chemotherapy. They were the first drugs in those relevant categories to demonstrate considerable clinical efficacy and broad use for TB. The basis of the new antimicrobial regimen for TB was ultimately established by initiating a regimen with INH, RIF, and PYR combination and continuing the regimen with INH and RIF [47].

CO-INFECTIONS

TB/HIV co-infection

TB and HIV are individually the two greatest ongoing public health threats globally. In combination, these two diseases can be even more destructive. There are approximately 100,000 individuals with HIV, and out of these patients, 10,715 are suffering from TB. HIV increases the risk of developing TB 26–36 times more than the non-infected people. Therefore, HIV infection is the leading risk factor for

enhancing the possibility of active TB from latent infection. Although other opportunistic infections occur mostly when the CD4+ lymphocytes count is less than 200 cells/mm3, active TB can occur throughout the course of HIV infection.

Clinical manifestations of TB among HIV subjects depend on the stage of HIV infection [48]. It has been shown that retroviral replication is up-regulated following the exposure of alveolar macrophages and lymphocytes from HIV patients to MTB *in vitro*. HIV replication in activated lymphocytes is increased by pleural fluid from patients with TB; in HIV patients with pulmonary TB. It is likely that TB increases HIV replication through the production of TNF-α, IL-1, and IL-6 following macrophage induction. According to clinical studies, TB exerts harmful effects on the course of HIV infection.

It has been reported that the mortality rate in TB/HIV co-infected patients was twofold higher than those without TB, which was not dependent on the CD4 cell count. The main predictor of survival in TB/HIV co-infected patients is the degree of immunosuppression because negative tuberculin skin test (TST), early opportunistic infections, and low CD4 cell counts are linked to increased mortality [49, 50].

Due to the associated immunosuppression of HIV, it is not easy to diagnose active TB because of a higher probability of atypical and extrapulmonary presentation and the weak performance of standard diagnostic tools. Although the gold standard method for TB diagnosis is still culturing, the alteration of the host immune response to MTB in HIV patients leading to diagnosis by microscopy and culture is hampered. Moreover, the increasing extrapulmonary TB rate in HIV patients is another challenge to TB management in countries without access to radiology tests [51].

It is believed that up to 50–60% of cases of pulmonary TB can be diagnosed by the sputum smear examination for acid-fast bacilli (AFB) in well-resourced laboratories. In poorer countries, the rates of AFB detection are lower due to inaccessibility to high-quality microscopy services. Furthermore, in countries with a high incidence of TB/HIV co-infection, the detection rate is even lower because pulmonary TB is naturally paucibacillary in HIV individuals [52].

Studies prior to the HIV epidemic reported 1.22 smear-negative and extrapulmonary TB cases for each smear-positive case. In countries with a high incidence of HIV infection, smear-negative TB is not diagnosed early. In one study in sub-Saharan Africa, it was reported that a third of patients with smear-negative TB passed away within a year of their first diagnosis. Recurrent pulmonary TB grew approximately in the third of the remaining individuals [53]. Atypical radiographic findings like infiltrates with no cavitation and hilar lymphadenopathy are likely observed in HIV patients. There is a link between the radiographic presentation and the CD4 lymphocyte count. The correct radiographic diagnosis will be more difficult due to the nonspecific findings of pulmonary infiltrates, in the middle or lower lobes, in TB/HIV co-infected people [54].

The additional key information in an HIV-infected child with TB is obtained by TST, particularly if there is not any positive contact history. Variable sensitivity restricts TST to diagnose TB in HIV-infected children. Severe malnutrition, severe TB disease, and HIV infection are the major clinical causes of false-negative results. Implementation is always challenging, particularly in peripheral health services, due to the costs, obtaining, and the necessity of cold chain storage. Hence, TST is not often present in resource-limited scenarios where TB/HIV is common [55, 56].

Kibiki et al. focused on TB and HIV endemic scenario and the impact of real-time PCR for MTB in bronchoalveolar lavage fluid; they reported that sputum of AFB smear and the serological test had sensitivities of 66.7 and 0%, in the respective order. PCR with CT 32 had a sensitivity of 85.7% and specificity of 90.9% to diagnose pulmonary TB in bronchoalveolar lavage. The development of active TB was not observed in any patient with positive PCR but negative culture during 18 months follow-up. AFB smear and serology were very low in terms of sensitivities in these HIV-infected patients. PCR of bronchoalveolar lavage (BAL) with CT value 32 had enhanced specificity to diagnose active pulmonary TB [57].

Comparisons among assays in high TB-HIV co-infection scenarios require optimizing the molecular assessment. Since extrapulmonary TB is commonly observed in patients with advanced immunosuppression, it is probable that more than half of them would be AFB smear-negative [58]. Treatment in TB/HIV co-infected patients poses the main challenge due to long-term high pill-burden regimens, interactions between TB medication and combination antiretroviral therapy, overlapping toxicity and adherence issues, high rates of MDR-TB, and injecting drug use [59]. TB/HIV co-infected patients need highly active antiretroviral therapy (HAART) to achieve an excellent long-term outcome. However, these patients experience a higher rate of adverse drug reactions to HAART [60].

Some studies suggest that up to 45% of patients with TB/HIV co-infection may be at risk of developing immune reconstitution inflammatory syndrome (IRIS) following the initiation of antiretroviral therapy (ART); risk factors for the development of IRIS include CD4 counts less than 100 and disseminated TB infection at baseline as well as rapid increases in absolute CD4 count following the initiation of ART. Thus, existing evidence suggested that IRIS is a common complication of combination antiretroviral and anti-TB therapy and raised the possibility that delaying ART until anti-TB therapy either in the continuation phase or even completed might be beneficial by reducing the infectious burden and risk of IRIS [61].

The rate of TB-IRIS incidence in co-infected patients is 7–36%. In the Asia-Pacific region, the rates of TB-IRIS among TB/HIV co-infected patients receiving ART under TB treatment were 26.2% in Thailand and 8% in India. IRIS presentations include unmasking of previously undiagnosed TB, paradoxical deterioration of pre-existing TB lesions, or the new lesions following ART initiation. Manifestations may include high fever, lymph node enlargement, deteriorating respiratory symptoms and signs, cold abscess formation, or deteriorating central nervous system lesions [7].

TB/HBV co-infection

Hepatitis B is a dangerous infection brought about by the hepatitis B virus (HBV); it targets the liver and is capable of causing both acute and chronic diseases. It is estimated that past or present HBV infections are found in two billion people; besides, an estimated 240 million people are chronically infected with HBV worldwide. Generally, about half of the global population resides in high hepatitis B endemicity areas. The worldwide occurrence of HBV infection is heterogeneous. The occurrence of hepatitis B is maximum in sub-Saharan Africa and East Asia, in which 5–10% of the grown-up population is chronically infected [62].

Anti-TB drug-induced hepatotoxicity increases the risk of HBV, which would lead to the cessation of treatment. Due to the likely co-existence of TB and hepatitis B in patients from endemic areas, some researchers recommend providing HBV testing to TB patients that could probably boost TB management and treatment (by careful checking and treatment modification when necessary). Hepatitis B might also influence patients with TB, but current data on HBV occurrence and risk factors among TB patients are scarce; data concerning hepatitis B occult and HBV genotypes targeting these patients are unavailable [63].

The problem of INH-induced hepatotoxicity in the treatment of LTBI in persons infected with hepatitis B has been investigated by a limited number of studies. A short study from Philadelphia among Southeast-Asian individuals could not differentiate the occurrence of INH hepatotoxicity between chronic hepatitis B carriers and non-carriers [64]. Another study of Vietnamese immigrants treated for LTBI with INH in Iowa and Illinois was able to discriminate between hepatitis B carriers with and without hepatitis B "e" antigen (HBeAg), a marker of active hepatitis B viral replication and related liver inflammation. Three of 21 (14%) individuals with HBeAg experienced symptomatic transaminase elevation of more than five times higher than normal while taking INH [65, 66].

TB/HCV co-infection

Hepatitis C virus (HCV) recently became an important global public health problem. Few studies have focused on the occurrence of HCV infection among patients internationally, and data on the rates of HCV co-infection among patients with TB are scarce [67]. In Georgia, a previous study reported a high prevalence (22%) of HCV infection among patients with TB. According to their results, HCV co-infection did not depend on the previous incarceration, tattoo, and previous sexually transmitted infections. Hepatotoxicity is the main negative effect of the three first-line anti-TB agents, including INH, RIF, and PYR [68].

It is likely that the risk of developing drug-induced hepatotoxicity is increased by the underlying liver disease may increase and HCV co-infection might increase the risk of anti-TB drug-induced hepatotoxicity [69]. Anti-TB drug-induced liver injury (DILI) is challenging in chronic liver disease, especially cirrhosis since it happens more commonly in this unique population leading to more severe outcomes in individuals with cirrhosis than those without cirrhosis. However, data on the occurrence and outcome of anti-TB DILI in advanced liver disease are scarce. According to Ungo et al., the occurrence of DILI was 24% in HCV-infected individuals receiving INH, RIF/rifabutin, and PYR in comparison with 5% in HCV-uninfected individuals [70].

DIAGNOSIS PROBLEMS

Diagnosis of latent TB infection

MTB-infected patients have host immune responses to mycobacterial antigens without any symptoms of TB disease. Much discussion about the actual state of latency and the degree of metabolic activity in TB-associated states. There is a continuum between latent TB infections (LTBI) and active TB rather than two distinct disease states. It is reported that approximately 10 million subjects in the US are infected, and more than two billion subjects have LTBI worldwide [71].

Due to the difficulties in diagnosing LTBI, these estimates are approximate. Detection of LTBI in immune-compromised patients is highly important due to their increased risk of progression to active disease. However, there is no definitive test to detect the MTB infection and current tests assess the host immune response to MTB without detecting the infection itself. Currently available tests to diagnose LTBI include the TST, the interferon-γ release assay (IGRA or QuantiFERON-TB Gold In-Tube assay), and the T-SPOT.TB test [72].

TST is widely used and inexpensive; however, it has three limitations, including poor specificity in BCG immunized people, cross-reaction with NTMs, and poor sensitivity in immune-compromised patients [73]. The sensitivity of TST is more than 95%, as measured in clinically well subjects with previously treated TB. False-negative reactions occur more frequently in infants and young children under eight weeks after infection, in patients with HIV or disseminated TB, and in subjects that recently received viral vaccines. The specificity of the TST is decreased among individuals vaccinated post infancy with BCG and those with repeated vaccination. Furthermore, people living in areas where NTMs are common have more false-positive TST reactions [74].

To surmount the TST limitations, there are numerous up-to-date skin tests and interferon-gamma release assays (IGRAs), including the Diaskintest, C-Tb skin test, EC-Test, and T-cell spot of the TB assay, QuantiFERON-TB Gold In-Tube, QuantiFERON-TB Gold-Plus, LIAISON QuantiFERON-TB Gold Plus test, and LIOFeron TB/LTBI [75].

In 2017, the IGRA, namely QuantiFERON-TB Gold Plus (QFT-Plus; Qiagen, Germantown, MD), received FDA and will replace the QFT-Gold In-Tube (QFT-GIT), the former version of the assay [76].

In a prospective cohort study performed by Abubakar et al., the prognostic value of IGRA and TST in predicting the development of active TB was evaluated. They reported

that positive results for T-SPOT.TB, TST-15, and QuantiFERON-TB Gold In-Tube were better predictors of progression than TST-10 and TST-5. Their results showed that IGRA- and/or TST-based strategies are the best tests for screening of the potential TB progression among high-risk groups [77]. However, there are few studies on predictive values of IGRA-based tests to predict the progress of LTBI. Recently, Agostinis et al. carried out a survey to investigate the IGRA for LTBI screening. They evaluated the association between demographic information and clinical manifestations, and predictors of accordance with IGRA in a TST-positive prisoners. They reported accordance between TST and QFT-GIT in approximately 30% of the prisoners. TST/QFT-GIT discordance has not been associated with disease during follow-up. According to their results, the low risk of disease progression in people with incompatibility recommends that IGRA-based screening is essential [78].

The main advantages of QuantiFERON-TB Gold include using specific MTB antigens, performing in a single visit, eliminating false-positive results in BCG-vaccinated persons, avoiding costs and toxicity associated with unnecessary treatment. Limitations of this test include the need for phlebotomy, cost, inconsistent test reproducibility, and complicated interpretation due to frequent conversions and reversions and lack of consensus on the thresholds [79]. The sensitivity of QuantiFERON-TB Gold assay is similar to TST and, in several evaluations, is better, particularly among immune-compromised patients [80].

TST and QuantiFERON-TB Gold assay can detect TB infection by measuring memory T cells showing the host's sensitization to MTB antigens. Due to T-SPOT.TB and QuantiFERON-TB Gold assays measure laboratory T cell immune responses or peripheral-blood mononuclear cell responses to MTB specific antigens (which are not found in NTMs and BCG, and there is no cross-reactivity with BCG and NTMs), specificity of these tests for MTB are higher than TST. The ability of TST and QuantiFERON-TB Gold to identify persons at a higher risk of progressing to active TB is poor. The researchers showed that the positive predictive value for developing active TB was 1.5% for TST and 2.7% for IGRAs [81].

Diagnosis of TB in HIV co-infected patients

TB-HIV co-infection remains a major global public health challenge. In 2015, there were an estimated 10.4 million new TB cases, of which 1.2 million were among HIV co-infected individuals. MTB is the most common opportunistic pathogen among HIV patients, and co-infected patients are at high risk of death. The high mortality rates of TB-HIV co-infected patients mandate that co-infected people be diagnosed as quickly as possible, and early diagnosis of both HIV and TB is critical to saving lives [82].

Diagnosis of TB in HIV co-infected patients is often difficult for several reasons, including atypical radiographic findings, resemblance to other opportunistic pulmonary infections, frequently negative sputum smears, and a higher rate of extrapulmonary TB. Less than half of TB cases in HIV-TB co-infected patients are diagnosed before death, and new assays must be done to decrease the delay in diagnosis of TB in HIV co-infected individuals [83].

The radiography of pulmonary TB is dependent on HIV immunodeficiency phases. In the early stage of HIV, in which persons are not immunosuppressed, the radiography shows typical lesions with or without cavities (similar to HIV-negative persons). At an advanced stage of HIV in which persons are immunosuppressed, the radiography reveals miliary TB, lymphadenopathy of mediastina, extra pulmonary involvement, and lower lobe infiltrate [84].

WHO recommends TB screening at the time that HIV is diagnosed and before the initiation of antiretroviral therapy. There is no globally accepted tool for TB screening in HIV-infected people. Although many evaluations are performed to develop a simple assay for ruling out TB in HIV-infected people, methodological issues preclude using any of these as the basis for global health policy [85]. Microscopic examination for acid-fast bacilli (AFB) is the most frequent assay to detect TB. Although this method is rapid, inexpensive, and easy to carry out, its sensitivity in TB-HIV co-infections is low. The sensitivity of microscopic examination of sputum for AFB in HIV infection is 43–51% and much lower in resource-limited settings with high rates of TB-HIV co-infection. Smear-negative TB exacerbates the difficulty of TB detection in HIV co-infected patients, leading to further delays while diagnosis or treatment evaluations are being done [86].

Culture and antibiotic susceptibility tests should be done to confirm the smear microscopy. This is even too serious in regions where MDR- and XDR-TB circulate since these methods would prevent the indiscriminate use of antibiotics and the further spread of resistant isolates [87]. Therefore, WHO recently suggested that high TB burden countries improve laboratories for culture, which would definitely increase TB diagnosis in HIV co-infected persons. Although culture and antibiotic susceptibility tests are the gold standards in TB diagnosis and follow-up, they can take up to 6–8 weeks. A false-negative result may be obtained for 10–20% of TB isolates. Furthermore, these tests are more expensive than microscopic examination, requiring extended incubation times, highly trained personnel, and specialized equipment. In addition, most of the world's poorly resourced regions with high TB burdens have very few reference laboratories capable of reliably performing these methods [88].

Nucleic acid amplification test (NAAT) is the only clinical diagnostic proven assay (aside from microscopy and culture) for MTB detection. The three most commonly used MTB NAAT methods include transcription-mediated amplification (Gen-Probe), polymerase chain reaction (PCR), and strand-displacement amplification (Becton Dickinson). Additionally, optimized versions of NAAT include fluorescence *in-situ* hybridization (FISH), loop-mediated isothermal amplification (LAMP), and Line-probe assays (LPAs). Despite the promising NAATs advantages, such as excellent specificity and sensitivity and rapid detection of smear-negative TB, due to their cost and complexity, NAATs have only limited use in endemic regions of TB [89].

TST is not highly sensitive to detect TB with impaired cell-mediated immunity, such as HIV patients (in spite of

the high sensitivity and specificity in LTBI). Because of LTBI identification, this limitation makes adopting strategies to reduce mortality rates in HIV-infected persons possible. It is necessary to perform TST in all HIV patients at the time of diagnosis and annually [90]. The risk of LTBI reactivation is up to 100-fold compared to HIV-uninfected persons, while developed active TB in the HIV individuals might have an incidence of 1.7–7.9 in 100 persons per year. Consequently, following a positive diagnosis result (in duration equal to or greater than 5 mm), the patient is required to receive INH chemoprophylaxis [91].

In low TB burden regions, IGRA is a promising alternative to TST for detecting TB infection. IGRA has greater specificity and sensitivity than TST for diagnosing TB infection and a better correlation with the intensity of exposure. Therefore, it is a promising option for better assessing TB infection in immunosuppressed persons, particularly HIV patients [92].

However, the use of IGRA in HIV-positive patients remains a matter of debate. Investigations of both IGRAs for LTBI diagnosis in HIV patients have shown conflicting results regarding the effect of CD4+ T cells. The T-SPOT.TB is thought to be less susceptible than QFT-IT to this impact as the number of peripheral mononuclear cells in the assay is standardized. The sensitivity of the QFT-IT is impaired in those with advanced immunosuppression; however, T-SPOT.TB sensitivity is impaired in those with low CD4+ T cell counts [93].

DIAGNOSIS OF MDR- AND XDR-TB

MDR-TB is defined as resistance to INH and RIF antibiotics in patients that have been infected with TB. Extensive drug-resistant TB (XDR-TB) is defined as resistance to at least four first-line and second-line antibiotics for TB. According to data, 5% of TB cases are estimated to have MDR-TB, 3.3% of MDR-TB cases are among newly treated TB cases, and 20% of them are among previously treated TB cases. Also, it is estimated that 9.5% of MDR-TB patients are XDR-TB [94]. Despite their recognized limitations, phenotypic drug susceptibility tests remain the gold standard for diagnosing MDR- and XDR-TB.

Liquid media are more commonly used for drug susceptibility tests than solid media in richly resourced countries. Traditional solid drug susceptibility tests are carried out on Lowenstein-Jensen or Middlebrook7H10/Middlebrook7H11 media. The original system using liquid media for drug susceptibility tests is the BACTEC MGIT 960 system, which used fluorescent light emission to detect TB growth and has more than 95% sensitivity and specificity for detection of resistance to INH and RIF (MDR-TB) [95].

Colorimetric methods are new rapid phenotypic methods that use the color change of tetrazolium bromide and resazurin dyes to diagnose MDR- and XDR-TB. Another novel, low-cost, rapid, simultaneous, economical, and highly sensitive/specific liquid culture method for diagnosis of MDR-TB is the microscopic observation of drug susceptibility (MODS assay), depends on faster growth of MTB in a liquid medium, and features cording colonies of MTB in a liquid medium can be visualized microscopically applying an inverted microscope [96].

Molecular assays for MDR- and XDR-TB identify fast-track the time from 6-8 weeks to 1–2 days. More than 95% of RIF -resistant MTB isolates have a mutation in the hot spot region of the *rpoB* gene, and Gene Xpert MTB/RIF assay works by detecting MTB and resistance to RIF [97]. WHO suggests that Xpert assay should be used as the initial diagnosis method in persons suspected of having TB-HIV co-infection and MDR- or XDR-TB. Due to the lack of accuracy of smear microscopy, it could also be used as a follow-on method to microscopic examination for AFB in settings where TB-HIV co-infection and MDR- or XDR-TB are of lesser concern (especially in smear-negative specimens) [98].

LPAs are great promising molecular assays to detect gene mutations that signal drug resistance. They are most suited for rapid diagnosis, which can be directly used on clinical specimens (such as sputum). LPAs are a family of novel DNA strip-based tests that use PCR and reverse hybridization methods to rapidly identify INH and RIF or fluoroquinolones (FLQs), ETB, and injectable agents. Currently, two-LPAs are commercially available, including INNO-LiPA Rif. TB and Genotype MT BDR plus assay [99, 100].

Sequencing-based molecular methods, including Sanger sequencing, pyrosequencing, and testing through the CDC molecular detection of drug resistance service, can be used to diagnose MDR- and XDR-TB. These methods have the advantage of reporting on the actual mutations, which can be helpful in interpretation. Although non-sequencing molecular methods (such as Line-probe and Gene Xpert MTB/RIF assays) can report silent mutations as antibiotic-resistant, the characterization of the mutation responsible for resistance to antibiotics is defined using a sequencing-based method. Therefore, after detecting antibiotic resistance using a non-sequencing assay in low-incidence regions, DNA sequencing is recommended for confirmation [96, 101, 102].

Diagnosis of TB in the pediatric population

TB is one of the 10 leading causes of death among children worldwide. There are more than 1 million TB cases among the child population, accounting for 130,000 deaths annually. The incidence of childhood TB is 10% of the total global TB incidence rate, but TB notification rates are lowest in children, with roughly 40% of incident cases in children younger than 15 years having been diagnosed and notified in 2015 [103]. Childhood TB is the result of contagious adult contact. TB-endemic areas have high rates of transmission because of high case density and lengthy diagnostic delay. Since childhood TB reflects ongoing transmission, children are influenced most acutely in areas in which an adult TB epidemic is not appropriately controlled [104].

In many scenarios, childhood TB will be undetected for some reasons such as the largely unidentified outcomes of children with TB, the effort to diagnose the pulmonary TB, insufficient methodical investigations on TB in children, and the belief that TB in children is not of importance for TB

control [105]. Additionally, collecting samples from young children for bacteriological diagnosis could not be done easily, and the paucibacillary nature of childhood TB means low yield from laboratory techniques such as smear microscopy. Finally, laboratory diagnosis with culture or Xpert MTB/RIF is typically unavailable in facilities in which children will be dealt with. Thus, many children with TB would go undetected [106].

The evaluation of TB in children is usually due to contact investigation or routine immigration screening or after presenting with findings indicative of TB [107]. There is no gold standard test for the diagnosis of TB in children, and the traditional and molecular bacteriologic tests used in adults are not specific and/or sensitive for childhood TB. In addition, analysis of the symptoms or signs of TB in the pediatric population is not easy, and younger children often have complex presentations with more severity and mortality [108].

Chest X-ray (CXR) is one of the most valuable methods for diagnosing TB in the pediatric population. Although chest radiography findings are diverse, pronounced hilar adenopathy is highly suggestive of TB [107]. TST and IGRA tests have insufficient data to be suggested as the diagnostic methods in MTB-infected children, and they cannot distinguish active TB infection from LTBI in these patients [74]. The gold standard for diagnosis of active TB in the pediatric population is the growth of MTB in culture [109].

TREATMENT PROBLEMS

Treatment of latent TB infection

The goal of treatment of LTBI is to eradicate existing asymptomatic infection to prevent disease and decrease the progress of TB infection to TB disease. This treatment is described as 'preventative therapy' or 'prophylaxis' [110]. The prophylactic treatment for LTBI played the main role in TB control worldwide during the last decade. The global strategy in treating TB in areas with a low incidence of TB is the preventive treatment of LTBI [111]. In addition, one of the main requirements to move towards TB elimination is prophylactic therapy to a broad group of latently infected individuals [112].

The medications used for the treatment of LTBI are also those used for active TB. However, not all effective agents against active TB are suitable for LTBI. All regimens for the treatment of LTBI require months of daily anti-TB drugs [113]. The Center for Disease Control and Prevention (CDC) recommends four treatment regimens for LTBI, including INH, rifapentine, or RIF. Since patients are more likely to complete shorter treatment regimens, healthcare providers should prescribe the more convenient and shorter regimens. In addition, because of the reports of severe liver injury and deaths, CDC suggested RIF- PYR combination should generally not be used to treat LTBI [114].

A once-daily regimen of INH consumed for 9 months is currently used as the standard LTBI treatment. In the USA, this is used to treat more than 80% of infections with LTBI. If the patient completes the treatment, 90% efficiency is expected. Nevertheless, less than half of the patients complete treatment; thus, the efficiency of this approach is decreased considerably [95]. Recommendations from the National Tuberculosis Controllers Association and CDC, 2020, are shown in Table 2.

Treatment of TB in HIV co-infected patients

HIV can increase the risk of poor TB treatment outcomes, including death, relapse, and acquired drug resistance to anti-TB drugs. These poor outcomes have increased concerns about the use of standard regimens for TB treatment in HIV-infected persons [115]. The treatment of LTBI can decrease the risk of active TB in patients with HIV, especially those with a positive TST [116].

In the study conducted by Cohen et al., the effects of INH preventive therapy for LTBI in HIV-TB co-infected patients were investigated. They reported that INH preventive therapy reduces TB incidence among these patients. Yet, in areas where re-infection is common, patients are likely to be re-infected after completing a single course of INH preventive therapy, and the treatment may fail to control TB [117].

Table 2. Recommendations from the National Tuberculosis Controllers Association and CDC

Priority rank*	Regimen	Recommendation (strong or conditional)	Evidence (high, moderate, low, or very low)
Preferred	3 Mos isoniazid plus rifapentine given once weekly	Conditional	Moderate
Preferred	4 Mos rifampin given daily	Strong	Moderate (HIV negative)
Preferred	3 Mos isoniazid plus rifampin given daily	Conditional	Very low (HIV negative)
		Conditional	Low (HIV positive)
Alternative	6 Mos isoniazid given daily	Strong	Moderate (HIV negative)
		Conditional	Moderate (HIV positive)
Alternative	9 Mos isoniazid given daily	Conditional	Moderate

Abbreviation: HIV = human immunodeficiency virus.

* *Preferred:* excellent tolerability and efficacy, shorter treatment duration, higher completion rates than longer regimens and therefore higher effectiveness; *alternative:* excellent efficacy but concerns regarding longer treatment duration, lower completion rates, and therefore lower effectiveness.

Akolo C et al. have studied the treatment of LTBI in HIV-infected patients and shown that preventive therapy versus placebo reduced the risk of active TB by 32%. Moreover, in TST-positive persons, preventive therapy reduced the risk of active TB by 62% [118]. The global recommendations and guidelines on the treatment of TB in HIV co-infected patients propose that the six-month treatment should be used for active TB. They state that periodic thrice-weekly dosing programs are good choices in patients infected with HIV, but WHO suggests two-time dosing for them [119]. In patients with HIV and active TB, antiretroviral therapy reduces the risk of TB relapse and has dramatic reductions in the progression to AIDS and death [117].

Treatment of MDR- and XDR-TB

Multi-drug treatment is one of the major challenges for TB control programs and the responsible factors for the emergence of MDR- and XDR-TB, including prolonged therapy, inadequate and incomplete treatment, low compliance, and stiff administration schedules. Therefore, an effective system is required to improve the effectiveness of anti-TB agents [120]. Due to the complex, toxic, and costly regimens for the affected patients with drug-resistant TB, the currently recommended treatment regimen is not tolerable for them. It is estimated that the cost of drugs to treat one MDR- and XDR-TB patient in Europe is 23,000 and 93,000 Euro, respectively. Such financial resources are not available for the majority of these patients, particularly in low- or middle-income countries [121].

Kaliakbarova et al., in their evaluations in Kazakhstan, found that psychosocial supports could improve the treatment adherence in patients infected with MDR-TB. They reported that 23% of these patients had defaulted on treatment due to financial constraints [122]. Sharma et al. focused on the quality of life of MDR-TB patients in north India and revealed that half of the patients did not start working even one year following the treatment. Therefore, their salary was reduced due to work absence, and they had to spend their income for treatment [123].

Probably, the drugs not taken previously by the case or persons with *in vitro* activity based on drug-susceptibility testing results could be used effectively for MDR-TB patients. Hence, an in-depth drug history is highly important to formulate the MDR-TB regimen [124]. According to the instructions for treating RIF-resistant TB, at least five effective TB medicines, including a newer-generation FLQ, second-line injectable drugs, and PYR, should be taken. Each of the five drugs in such a regimen is beneficial [125].

CLO is a tremendously important antibiotic for MDR/XDR TB therapy. The studies reported that the treatment arm containing CLO, increased therapy success rate, accelerated sputum culture conversion, and accelerated cavity closure were shown. In terms of negative side effects, skin pigmentation has been observed in 75–100% of cases within a few weeks [126]. Linezolid (LNZ) is an important anti-TB agent to treat infection with MDR- and XDR-TB, and its suitable activity have been confirmed *in vitro* and animal studies. Yet, it causes severe side-effects such as myelosuppression, peripheral neuropathy, and lactic acidosis; it is recommended to stop the usage upon serious adverse effects arise [127].

The use of BDQ to treat pulmonary TB in adults with MDR-MTB strains was approved by the FDA a short time ago. Both dormant and actively replicating mycobacteria are killed by BDQ by inhibiting mycobacterial adenosine triphosphate synthase, interrupting energy production, and disturbing intracellular metabolism. It is recommended to reserve BDQ for patients with MDR-TB upon lack of an efficient regimen with PYR. It is also recommended to treat MDR-TB with documented resistance to any FLQs [128].

Treatment of TB in the pediatric population

It is believed that childhood TB is considerably linked to the worldwide TB case load (15–20% of cases), particularly in Africa, where TB is considered a major respiratory cause of death in children. The ongoing TB transmission and increased vulnerability because of HIV-induced immune compromise lead to a high disease burden [129].

According to the observational data in children, a 99% cure rate has been achieved by drug-susceptible pulmonary TB, 6 months' INH, and RIF combined with 2 months' PYR primarily. Nevertheless, quadruple therapy by adding ETB or STR in the intensive phase is usually suggested upon the high risk of INH-drug resistance [130]. According to WHO suggestions, all HIV-positive children should undergo a four-drug TB treatment, regardless of the TB disease severity (INH, RIF, PYR, and ETB), and it is necessary to avoid that intermittent therapy. Besides, it has been suggested to begin ART upon the tolerance to treatment and within 2–8 weeks after starting it [75].

CONCLUSION/OUTLOOK

Multidrug-resistant TB (MDR-TB) has increasingly grown in developing and industrialized countries over the past few years. The global growth of TB and the rapid emergence of MDR-TB underlies the need for developing new antituberculous agents and recent protocols for the efficient clinical control of TB patients using common anti-mycobacterial agents. During the 40 years after the release of rifampicin, no novel drugs, regardless of rifabutin and rifapentine, have been developed to control TB in the US and other countries.

The use of INH and rifampin alone or their combinations, as well as the INH and rifapentine combination, are available regimens for the treatment of LTBI. Unlike INH monotherapy, RIF-based treatments are shorter and better tolerated; therefore, they are prominent tools for inhibiting TB disease and eradicating the TB epidemic. For TB prevention, new vaccine approaches, host immunity-directed treatments, and ultra-short regimens with antibiotics are under assessment.

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regimens for the treatment of LTBI. Unlike INH monotherapy, RIF-based treatments are shorter and better tolerated; therefore, they are prominent tools for inhibiting TB disease and eradicating the TB epidemic. For TB prevention, new vaccine approaches, host immunity-directed treatments, and ultra-short regimens with antibiotics are under assessment.

Notwithstanding an urgent need to develop novel antimicrobial drugs and new protocols for administrating effective drugs against stubborn mycobacterial infections, no new drugs are progressing to solve this problem.

For diagnosis of TB, direct smear examination of sputum is the most common strategy, though culture is desirable if resources permit. For re-treatment purposes, reliable susceptibility testing is favorable, but it is costly that few developing countries can afford. Rapid culture testing and susceptibility testing are broadly available in wealthier nations. Molecular methods, such as polymerase chain reaction, DNA and RNA probes, and γ interferon tests, are rapid, sensitive, and specific tests for *M.tuberculosis*, but they are expensive and technically demanding. These methods are most helpful to diagnose multi-drug resistant organisms rapidly and to differentiate *M. tuberculosis* from other non-infectious mycobacterial species.

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