Drug-Resistant Tuberculosis

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Abstract The incidence of drug-resistant tuberculosis (TB), particularly multidrug-resistant TB and extensively drug-resistant TB, is increasing and is a major complication in global attempts to control TB. New anti-TB drugs and rapid diagnostics have been developed; however, the pathogenesis of drug resistance remains unclear. Fragmented treatment regimens, efflux pumps, and pharmacokinetic variability may all play a part in the rise of drug-resistant pathogens. Drug-resistant TB continues to be associated with poor treatment outcomes and high mortality rates.

1 Introduction

Tuberculosis (TB) is an ancient disease and one of the world's deadliest communicable diseases. The World Health Organization (WHO) TB estimates for 2013 included approximately 9.0 million cases and 1.5 million deaths. Although global rates of new TB cases have been decreasing since 2005, cases of multidrug-resistant TB (MDR TB) and extensively drug-resistant TB (XDR TB) have been increasing and are out of control in some regions, including Africa. Drug-resistant TB is currently one of the most important threats to global control of the disease (Dye et al. 2002). The proportion of MDR TB is higher among people who have been treated previously (20.5 %) and lower among new cases (<3 %). Eastern European and central Asian countries have the highest levels of MDR TB: 35 % of new cases and 75 % of previously treated cases. Drug-resistance surveillance data from 108 of 144 countries (75 %) indicate that approximately 480,000 individuals developed MDR TB in 2013. Furthermore, approximately 9 % of MDR TB cases were actually

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XDR TB. More than half of these MDR TB cases were estimated to have occurred in India, China, and the Russian Federation.

Mortality rates for untreated TB are high. Tiemersma et al. (2011) conducted a natural history study of TB and found that around 70 % of HIV-negative patients with sputum smear-positive pulmonary TB and 20 % of patients with culture-positive (but smear-negative) TB died within 10 years.

According to WHO, satisfactory treatment of XDR TB requires an 8-month intensive phase and a 20-month minimum overall treatment duration. Treatment regimens including second-line drugs are more toxic, more expensive, and less convenient than the standard anti-TB regimen. Drug-resistant TB, particularly MDR TB and XDR TB, requires a longer duration of treatment and has worse outcomes than drug-susceptible TB (Dheda et al. 2010a; Jacobson et al. 2010; Kvasnovsky et al. 2011). For instance, only 48 % of patients with MDR TB are treated successfully, with the disease incurring mortality rates of 15 % and a rate of lost of 28 %. Treatment outcomes for patients with XDR TB are worse, with a success rate of 33 % and a mortality rate of 26 % (WHO Global Tuberculosis Report 2013).

Analysis suggests the proportion of new MDR TB cases remained at approximately 3.5 % over the period 2008–2013. However, although drug-resistant TB, especially MDR TB and XDR TB, represents a small proportion of the patients with TB in countries with a high TB burden, these patients consume a large proportion of TB-control resources. In South Africa, the per-patient cost of treating XDR TB was US $26,392 (in 2011), four times greater than that for MDR TB and 103 times greater than that for drug-sensitive TB. Although drug-resistant TB represents only a fraction (2.2 %) of the total case burden, it consumes 32 % of the total estimated national TB budget in South Africa (Pooran et al. 2013). This disproportionate amount of total TB costs is due to the high cost of managing drug-resistant TB, high drug prices (Kang et al. 2006), implementation of the new Xpert MTB/RIF assay as the primary TB diagnostic test (Theron et al. 2011), and the need for extensive supervised patient care, all of which are likely to increase substantially.

2 Pathogenesis and Mechanisms of Drug-Resistant Tuberculosis (TB)

Drug-resistant TB develops in two ways. The first is primary or initial drug resistance: an individual is infected with a strain of *M. tuberculosis* that is already drug resistant. This kind of infection usually occurs in regions with a high prevalence of drug-resistant TB. The second is acquired or secondary drug resistance: resistance to the TB treatment develops as a result of inadequate or incorrect treatment regimens, efflux pumps, and genotype. Treating drug-susceptible TB with monotherapy increases the risk of drug-resistant mutations being selected and eventually becoming the dominant strain.

2.1 Selection of Drug Resistance

Spontaneous gene mutations of chromosomes that encode the target of anti-TB drugs and related *M. tuberculosis* metabolic enzymes are an important cause of single-drug resistance, and MDR is due to a variety of these drug-target gene mutations occurring. Luria and Delbruck (1943) famously showed that resistance-related *M. tuberculosis* genetic mutations were independent of selection pressure. Although the rate of spontaneous mutation is low in individual patients, there is concern that, given the large bacterial burden of up to 10^9 units and the level of bacterial replication, pre-existing *M. tuberculosis* resistant to one anti-TB drug may be possible in some patients.

The probability of pre-existing drug resistance to two or three anti-TB drugs is very small. However, resistance can be acquired, for example, if a patient receives long-term monotherapy, does not comply with treatment regimens, or receives a drug combination that is a pharmacokinetic mismatch (e.g., drugs in the combination have markedly different pharmacokinetic half-lives). The pharmacokinetic mismatch between rifapentine and isoniazid is thought to be a reason for the high rate of acquired rifamycin resistance among patients co-infected with TB and HIV who were treated with once-weekly rifapentine and isoniazid (Vernon et al. 1999). In this situation, patients actually received monotherapy. The initial drugs killed most of the susceptible *M. tuberculosis* subpopulation, and the pre-existing drug-resistant subpopulation was then able to replicate, eventually replacing the drug-resistant population. This form of resistance is known as acquired resistance, and accumulation of acquired resistance may cause MDR and XDR TB.

2.2 Acquired Drug Resistance Based on Drug Concentrations and Efflux Pumps

In some cases, drug-sensitive TB will progress to drug-resistant TB even if patients adhere to treatment (Calver et al. 2010). Some studies have shown that patients with low serum levels of isoniazid and rifampin may have a longer time to culture conversion and a worse overall treatment outcome, with the low concentration of anti-TB drugs possibly playing a role in acquired drug resistance (Blumberg et al. 2003; Jayaram et al. 2004; Weiner et al. 2005; Park et al. 2015). Studies using the hollow fiber system model of TB have shown that when one fluoroquinolone drug is used to kill *M. tuberculosis*, the bacteria easily develop resistance to that drug despite the drug concentration being much higher than the minimum inhibitory concentration (Gumbo et al. 2004, 2005). Other studies using the hollow fiber system model of TB also showed rapid development of resistance to isoniazid, rifampin, pyrazinamide, and ethambutol (Gumbo et al. 2007a, b, 2009). Acquired drug resistance is associated not only with the area under the curve but also with peak drug concentrations. Pasipanodya et al. (2012) conducted a meta-analysis and

found that the faster the isoniazid underwent acetylation, the higher the rate of acquired drug resistance. Another study found that drug concentrations and pharmacokinetics varied widely between patients. Among a sample of 142 patients, the ratio of the highest to lowest dose for isoniazid, rifampin, and pyrazinamide was 2.7; the ratios of the highest peak concentration to lowest concentration was 102 for rifampin, 31 for isoniazid, and 63 for pyrazinamide, and peak drug concentration and the area under the curve predicted more than 91 % of treatment failures. Patients with low rifampin and isoniazid peaks and area under the curve concentrations developed acquired drug resistance (Pasipanodya et al. 2013).

Acquired drug resistance is also associated with many resistance efflux pumps, which can protect *M. tuberculosis* replication and enable generation of chromosomal mutations (Srivastava et al. 2010; Pasipanodya and Gumbo 2011). Dose-scheduling studies found that once-weekly therapy regimens, which are associated with more abrupt changes in drug concentrations than are regular daily therapy regimens, were associated with efflux pump-related resistance. Efflux pumps are also a cause of clinically relevant *M. tuberculosis* drug resistance (Jiang et al. 2008; Spies et al. 2008).

2.3 Drug-Resistant TB Genotypes

Given *M. tuberculosis* has a low mutation rate and a slow replication rate, it is unclear how *M. tuberculosis* acquires resistance to multiple anti-TB drugs, especially under treatment with multiple drugs. The target encoding gene mutation of clinical drug-resistant *M. tuberculosis* isolates is closely related to drug resistance. The biological variability of *M. tuberculosis* is the main molecular cause of drug resistance; Table 1 shows the *M. tuberculosis* genes that are resistant to common anti-TB drugs. Recently, whole genome sequencing of clinical *M. tuberculosis* isolates has revealed the importance of mutation in the emergence of drug resistance (Ioerger et al. 2010; Casali et al. 2012). Sequencing of *M. tuberculosis* from patients for whom drug treatment failed revealed that multiple new drug-resistance mutations can occur (Sun et al. 2012). Multidrug resistance may pre-exist in some patients who were initially infected with a drug-susceptible strain of *M. tuberculosis* (Ford et al. 2013). Moreover, several studies have suggested that certain strains of *M. tuberculosis* may be associated with multi-drug resistance (Borrell and Gagneux 2009). Some drug-susceptible TB treated with 'DOTS' (directly observed treatment, short-course) progressed to MDR TB, which might be due to hypermutable *M. tuberculosis* strains in patients who also rapidly metabolize first-line drugs (Gumbo 2013). Mutation rates can differ both between and within genotypes, and the reasons for this are unclear. Some whole genome sequencing studies have shown that target-encoding mutations are relative to compensatory mutations in the *M. tuberculosis* genome (Comas et al. 2012; Sun et al. 2012). It is possible that the drug-resistance encoding mutation could affect both the strain structure and the antigen of *M. tuberculosis*.

Table 1 Genetic mutations related to drug-resistant *Mycobacterium tuberculosis*

Anti-tuberculosis drug	Mutated gene	Minimum inhibitory concentration	Percentage of mutation	Gene product
Isoniazid	*katG* *inhA*	0.02–0.2	50–95	Catalase peroxidase reductase analog
Rifampin	*rpoB*	0.05–1	95	Subunit of RNA polymerase
Pyrazinamide	*pncA*	16–50(PH 5.5)	72–97	Pyrazinamidase
Ethambutol	*embB*	1–5	47–65	Arabinosyltransferase
Streptomycin	*RpsL rrs* *gidB*	2–8	52–59; 8–21	Ribosomal protein S12 16S rRNA
Amikacin	*rrs*	2–4	76	16S rRNA
Capreomycin	*tlyA*			Methyl transferase
Fluoroquinolones	*gyrA* *gyrB*	0.5–2.5	75–94	DNA gyrase A subunit
Ethionamide	*etsA* *etsB*	2.5–10	37	Nitric oxide
Paminosalicylic acid	*inhA* *thyA*	1–8	36–56	Synthesis of thymidine

3 Diagnosis of Drug-Resistant TB

Laboratory testing is important for the confirmation of TB, especially drug-resistant TB. The identification of drug-resistant TB needs to detect *M. tuberculosis*, culture it, and then identify the bacterial species and strains. A drug-sensitivity test (DST) is then conducted using either liquid or solid methods or a WHO-approved molecular method.

3.1 Definitions of Drug-Resistant TB (WHO, 2013)

Mono-resistance: resistance to one first-line anti-TB drug only.

Poly-resistance: resistance to more than one first-line anti-TB drug, other than both isoniazid and rifampin.

MDR: resistance to at least isoniazid and rifampin.

XDR: resistance to any fluoroquinolone and at least one of three injectable second-line drugs (capreomycin, kanamycin, and amikacin), in addition to multidrug-resistance.

Rifampin resistance: resistance to rifampin detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. It includes any resistance to rifampin, whether mono-resistance, poly-resistance, MDR, or XDR.

3.2 *Phenotypic Drug Sensitivity Test (DST)*

3.2.1 Liquid and Solid Methods

Solid DST methods can be used for sputum, other body fluids, and other samples to detect different concentrations of first-line and second-line anti-TB drugs. However, the specificity of the solid method is low, the process is complex, and biological security is difficult to maintain. These problems limit the clinical application of the conventional solid methods (Martin et al. 2008; Visalakshi et al. 2010).

The liquid method can shorten the detection time but still needs 4–6 weeks to obtain a DST result, and the instrument and reagents are expensive (van Kampen et al. 2010).

3.2.2 Drug-Resistance Test by Phage

Subramanyam et al. 2013 reported that the sensitivity and specificity of phage lysin in detecting *M. tuberculosis* from sputum specimens was 90 % and 81 %, respectively, compared with conventional Lowenstein–Jensen (LJ) medium. The agreement between the methods was 87 %, and the rate of contamination was 9.3 %

3.2.3 Microscopic Observation Drug Susceptibility

Microscopic observation drug susceptibility (MODS) entails using an inverted microscope to identify bacteria by observing the structure of the strain in the liquid medium. Anti-TB drugs can be added to the liquid and the DST completed directly. Agarwal et al. 2014 reported that the identification rate between the MODS assay and the reference solid LJ/liquid mycobacteria growth indicator tube (MGIT) culture was 94.8 % (95 % confidence interval 92.3–96.5). Huang et al. (2013) reported that the sensitivity and specificity of the MODS assay to detect resistance to pyrazinamide were 97.8 % and 96.5 %, respectively. MODS is the best method with which to detect pyrazinamide-resistant TB in resource-limited regions.

3.3 *Genotype DST*

The molecular DST (MDST) provides a rapid TB diagnosis and detection of drug resistance with satisfactory sensitivity and specificity. These new molecular tests can detect TB drug resistance within 2 h. The Xpert MTB/RIF assay is a new test that can detect whether the TB is active and whether the *M. tuberculosis* is resistant to rifampin.

3.3.1 Line Probe Assay

The GenoType MTBDRplus assay can rapidly detect *M. tuberculosis* genes that confer resistance to rifampicin and isoniazid; both sensitivity and specificity are satisfied (Crudu et al. 2012; Raveendran et al. 2012; Aubry et al. 2014). WHO recommends this testing for the detection of MDR TB.

3.3.2 Xpert MTB/RIF Assay

The Xpert MTB/RIF assay can both detect *M. tuberculosis* and complete DST for rifampin within 2 h. Its advantages include that it deals directly with sputum specimens, it avoids contamination and biological hazards, and the operation process is simple (Menzies et al. 2012). The Xpert MTB/RIF assay is validated for sputum, and research indicates it can be used to diagnosis extra-pulmonary TB (Causse et al. 2011; Hillemann et al. 2011; Vadwai et al. 2011; Biadglegne et al. 2014).

4 Therapy of Drug-Resistant TB

To gain worldwide control of TB, treatments for drug-resistant TB, especially MDR TB and XDR TB, are urgently needed. Treatment strategies for drug-resistant TB should be based on the specific drug resistance and treatment history, among others. Treatment for drug-resistant TB currently involves an integrated strategy that includes chemotherapy, immunotherapy, interventional therapy, surgery, traditional Chinese medicine, and nutritional support.

4.1 Chemotherapy for Drug-Resistant TB

Chemotherapy remains the primary treatment for drug-resistant TB. The chemotherapy regimen should be based on anti-TB medication history, drug resistance, and the prevalence of *M. tuberculosis* strains in the region.

Mono-resistant TB often involves initial drug resistance or primary drug-resistant TB, and the standard chemotherapy for the particular category of TB will be effective. However, the lack of four effective core drug combinations within the standard chemotherapy regimens means the potential does exist for the cure rate to decrease or the relapse rate to increase. As a result, especially for mono-resistance to rifampin, the chemotherapy regimen should be adjusted appropriately to avoid the possibility of treatment failure and the risk of acquired drug resistance.

MDR or poly-resistant TB are both more complex than mono-resistant TB. Drug resistance takes many forms but usually falls into one of three combinations: resistance to two drugs, resistance to three drugs, or resistance to four drugs. Patients with TB treated with the standard chemotherapy regimen are at greater risk for MDR TB, and treatment regimens should be adjusted to ensure patients receive four drugs that are effective or to which the TB is likely susceptible.

WHO (2011) recommends three basic treatment strategies for MDR TB—standardized, individualized, or empirical—as outlined in the following sections.

4.1.1 Standardized Treatment

Standardized treatment is a group of treatment regimens designed according to DST information and categories of patients within a country or region; patients with the same type of disease should be treated with the same treatment regimen within a country or region.

4.1.2 Individualized Treatment

Individualized treatment is based on the history of anti-TB treatment received and DST results for each patient (often DST is conducted for both first- and second-line drugs). Different patients should receive different individualized treatment regimens.

4.1.3 Empirical Treatment

Each patient's treatment regimen should be determined according to their anti-TB medication history and the DST of a country or region. The treatment regimen should be adjusted according to DST results (often DST is conducted only for a limited number of drugs). This type of treatment is mainly suitable for regions in which individual DST is not available. The basic strategy also applies to other types of drug-resistant TB.

The principles for the treatment of MDR TB with chemotherapy are as follows: (1) Regimens include at least four drugs to which the isolate is (or probably is) susceptible. (2) Regimens include a later-generation fluoroquinolone (e.g., moxifloxacin or levofloxacin) plus an injectable drug (e.g., amikacin or kanamycin), any first-line drug to which the isolate is susceptible, and a fourth drug (e.g., cycloserine, terizidone, ethionamide). (3) Injectable drugs are used for at least 6 months, and the total duration of treatment is 18–24 months.

Effective chemotherapy for the treatment of XDR TB is still lacking. Treatment is often based on nutritional support, symptom relief, improving respiratory function, and other measures to control infection with other pathogens. For disease with low-level resistance to a fluoroquinolone but sensitive to a later-generation

Table 2 Alternative grouping of anti-tuberculosis agents (2011)

Grouping	Drugs
Group 1: first-line oral agents	Isoniazid (H); rifampin (R); ethambutol (E); pyrazinamide (Z); rifabutin (Rfb)
Group 2: injectable agents	Kanamycin (Km); amikacin (Am); capreomycin (Cm); streptomycin (S)
Group 3: fluoroquinolones	Moxifloxacin (Mfx); levofloxacin (Lfx); ofloxacin (Ofx)
Group 4: oral bacteriostatic second-line agents	Ethionamide (Eto); protionamide (Pto); cycloserine (Cs); terizidone (Trd); P-aminosalicylic acid (PAS)
Group 5: agents with unclear efficacy (not recommended by WHO for routine use in patients with MDR TB)	Clofazimine (Cfz); linezolid (Lzd); amoxicillin/clavulanate (Amx/Clv); thioacetazone (Thz); imipenem/cilastatin (Ipm/Cln); high-dose isoniazid (high-dose H); clarithromycin (Clr)

fluoroquinolone (moxifloxacin is often used even when a DST indicates resistance to fluoroquinolones) (Jacobson et al. 2010) and possibly sensitive to a drug such as amikacin or capreomycin (administered via injection), the anti-TB treatment regimen could consist of the above-mentioned later-generation Fluoroquinolone, Amikacin, or Capreomycin and two drugs from the fifth group of anti-TB drugs (see Table 2). Linezolid and bedaquiline may shorten the time of sputum negative conversion for patients with XDR TB, but the cost and toxic effects are significant issues (Lee et al. 2012; Worley and Estrada 2014; Guglielmetti et al. 2015).

4.2 Immune Therapy

About 20 % of TB cases self-cured before the age of anti-TB chemotherapy, which supports the theory of immune-mediated clearance of *M. tuberculosis*. Some studies have reported immune-mediated clearance (Eum et al. 2010; Basile et al. 2011; Lindau et al. 2013). The most two active and acceptable immune agents are cytokine and Mycobacterium vaccine.

Many studies have shown the ability of immunomodulatory drugs to improve TB treatment outcomes (Dlugovitzky et al. 2006; Dheda et al. 2010b; Faujdar et al. 2011; Gao et al. 2011; Yang et al. 2011; Butov et al. 2012; Gupta et al. 2012a, b; Skrahin et al. 2014). Immunomodulatory drugs currently in clinical use include mycobacterium vaccae, interferon-γ, recombinant human interleukin 2, steroids, and tumor necrosis factor antagonists, among others. Immune agents are not recommended for patients with mono-resistant TB who are in good physical condition. Patients in poor physical condition can be treated with one kind of immune agent. Patients with MDR TB or XDR TB can be treated with one or two select immune agents depending on their physical and financial status.

4.3 Interventional Therapy

The widespread clinical use of bronchoscopy in recent years means anti-TB drugs administered via percutaneous lung puncture or bronchoscopy have become an effective treatment method for drug-resistant TB, particularly MDR TB.

Interventional therapy is gradually being used as a supplementary treatment method to cure drug-resistant TB (Yang et al. 2012), and we suggest that, as long as conditions permit, interventional therapy should be used as early as possible for drug-resistant TB, particularly MDR TB.

4.4 Surgical Therapy of Drug-Resistant TB

In the past 10 years, the increase in drug-resistant TB has seen a corresponding increase in the number of patients requiring surgical treatment. Surgical therapy has become more important in the treatment of drug-resistant TB, especially MDR TB.

The current theory for surgical therapy is that, for MDR TB, as long as lesions or cavities are confined to one lung or a lung lobe, surgery should be undertaken early to ensure a high cure rate and the lowest possible spread rate (Branscheid et al. 2003; Cummings et al. 2012; Suarez-Garcia and Noguerado 2012; Weyant and Mitchell 2012; Marrone et al. 2013; Calligaro et al. 2014; Mordant et al. 2014). However, surgery is not the end therapy for MDR TB. Generally, patients with MDR TB should receive more than 2 months of anti-TB chemotherapy before surgery as the chemotherapy could reduce spread to the surrounding lung tissue. Patients still require 12–24 months of chemotherapy after surgery.

4.5 Traditional Chinese Medicine Treatment and Nutrition Support

Traditional Chinese medicine can improve the immune function, physical condition, and clinical symptoms of patients with drug-resistant TB (Wang et al. 2015). Treatment of drug-resistant TB can lead to malnutrition, which can lead to worsening of drug-resistant TB. Therefore, patients with drug-resistant TB require nutritional support (Chisti et al. 2013; Hood 2013).

5 Perspectives

The early diagnosis of TB and drug-resistant TB is necessary for the global control of this disease. The slow growth of *M. tuberculosis* is the greatest obstacle to rapid diagnosis and DST. The further development of diagnostic tools, especially molecular methods, mean rapid detection of *M. tuberculosis* and specific chromosome mutations associated with phenotypic resistance to treatment will be possible. However, the cost efficiency of and appropriate settings for these new molecular methods will limit their clinical use. We hope more new laboratory tests and anti-TB drugs will be used clinically to improve drug-resistant TB-related mortality rates and treatment outcomes.

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