CHAPTER

26

Drug Resistant Tuberculosis

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INTRODUCTION

Drug resistant tuberculosis (DRTB) has hit the tuberculosis control programs like a thunderbolt. It has challenged all the aspects of tuberculosis management, from diagnosis till prognosis. India has had a major contribution in DR TB worldwide, with Mumbai city being the niche for DRTB strains. DRTB has emerged distinctly as a "Creation of mankind" than solely as a "Weapon of God" to curtail the ongoing population explosion. Emergence of resistance has been multi-factorial. Thus; to handle this roaring health hazard; fine tuning of ongoing programs, innovations with newer modalities, response estimation and foresight of future aspect should be dealt with by experts with experience and followed by the rest.

DEFINITION REVIEW

Drug resistant tuberculosis is defined as a documented microbiological confirmation of resistance of an isolate of acid fast bacilli to the standard first line and /or second line anti tubercular drugs, by an accredited laboratory.

- Monoresistance: Resistance to a single anti tubercular agent only.
- Poly-drug resistance: Resistance to more than one of first line anti tubercular drugs(except both rifampicin and isoniazid)
- Rifampicin resistance: Resistance to Rifampicin only either by phenotypic or genotypic measures. As most of the rifampicin resistant strains show an associated resistance to Isoniazid as well, it has been accepted as a surrogate marker of associated Isoniazid resistance too for treatment purposes.
- Multi-drug resistance (MDR TB): Resistance to Isoniazid and Rifampicin.
- Pre – extensive drug resistance (Pre XDR TB): An MDR resistance pattern with an additional resistance to either a Fluoroquinolone or any of the second line anti tubercular drugs (Amikacin, Kanamycin, Capreomycin).
- Extensive drug resistance (XDR TB): An MDR resistance pattern with an additional resistance to any fluroquinolone and to at least one of the three second line anti tubercular drugs (Amikacin, Kanamycin, Capreomycin).
- Total Drug resistance (TDR TB): Resistance to all the standard antitubercular agents, against which resistance is tested in laboratories. However, the word "total" may be a misnomer as there are drugs available having activity against tubercle bacilli but are not routinely tested like cycloserine, terizidone and class V anti TB drugs like amoxicillin-clavulanate, imipenem/cilastin, high dose isoniazid, clarithromycin, thiacetazone.

Table 1: Epidemiology of tuberculosis worldwide and in India

	Incidence	Prevalence	Mortality
Global	9.6 million (176/lakh/ year)	13 million (227/lakh/year)	1.1 million (21/lakh/year)
India	2.2 million (167/lakh/ year)	2.5 million (195/lakh/year)	2.2 lakhs (17/ lakh/year)

Source. *Global TB Report 2015*

EPIDEMIOLOGY (TABLE 1)

"We had it, we have it and we are embracing more"

STATISTICS

Global

- 3.3% of new TB cases and 20% of previously treated cases were found to have MDR-TB
- 58% of previously treated patients and 12% of new cases were diagnosed in 2014.
- 54% of MDR is harboring in India, China and the Russian Federation.
- A total of 111 000 people started MDR-TB treatment in 2014, an increase of 14% compared with 2013.
- Only 50% of MDR-TB patients were successfully treated. Approximately, 190 000 people lost their lives to MDR-TB in 2014.
- Extensively drug-resistant TB (XDR-TB) had been reported from 105 countries by 2015. Also, 9.7% of MDR-TB were turned into XDR-TB.

INDIA

- One fourth of global TB burden is born by India
- India has highest burden of TB, MDR TB patients and second highest load of TB HIV patients as Glob al TB report of 2015
- 71,000 MDR TB emerges every year from pulmonary TB patients.
- In India, Mumbai harbors the maximum load of

Table 2: Gene targets for drug resistance of anti-tubercular agents

Anti-tubercular drugs	Resistant gene
Rifampicin	RNA polymerase subunit B (rpoB)
Isoniazid	Catalase –peroxidase (katG) Enoyl acyl carrier protein(acp) reductase (inhA), Oxidative stress regulator (oxyR) Alkyl hydroperoxidase reductase (ahpC) Ketocyl acyl carrier protein synthase (KasA)
Pyrazinamide	Pyrazinamidase (pncA)
Ethambutol	Arabinosyl transferase (emb A, emb B, emb C)
Streptomycin	16 s ribosomal RNS (rrs), Ribosomal protein subunit 12 (rpsL)
Fluoroquinilones	Gyrase (gyrA, gyrB)
Ethionamide	Enoyl acyl carrier protein(acp) reductase (InhA)
Aminoglycoside	Phosphotransferase gene(strA)
Capreomycin	Haemolysin (tlyA)
Kanamycin	16S ribosomal RNA (*rrs*) gene

MDR-TB, seen in 24%–30% of new cases and 11%–67% of previously treated cases.

RISK FACTORS

Pathogen factor

An inherent resistance of acid fast bacilli (AFB) to an agent may exist, which is called Primary resistance. A concept of subpopulation has been known; wherein strains undergo spontaneous mutation while division, leading to primary resistance of varied pattern. Additionally, if a single anti TB medication is administered in the therapy or the regimen is inappropriate as leads to Secondary drug resistance. Patients having Secondary drug resistance can spread resistant disease to contacts that can develop primary drug resistant disease in them.

It has been said that it is the spontaneous chromosomal mutations occurring in M tuberculosis that confer resistance to anti TB drugs. It is known that a TB cavity contains 10^7 to 10^9 bacilli. Also known is the fact that Isoniazid resistance occurs in about 1 in 10^6 and Rifampicin resistance in about 1 in 10^8 replications. Thus the probability of spontaneous mutations causing resistance to both Isoniazid and Rifampicin would be 1 in 10^{14} replications. This calculation makes it clear, that as these many number of bacilli cannot be found even in patients with extensive cavitary pulmonary TB, the chance of the development of resistance to both Rifampicin and Isoniazid is very remote. Hence the increasing number of MDR- TB patients in the country is likely due to failure of National TB control program.

We have listed in Table 2 below the individual drug target genes that are responsible for the development of anti-TB drug resistance. Rifampicin & Isoniazid resistance development are both very different. For example, as contrast to Isoniazid which has got multiple genes involved in its resistance development; Rifampicin has got predominantly 1 gene involvement (>95%).

Immunology of MDR TB

Immunology of Tuberculosis has always been a matter of great interest & simultaneously a big mystery. What is it that exactly makes these patients resistant to drugs (apart from the genetic resistance)? Are there any inflammatory cells or cytokines involved? Are there any cellular deficiencies which predispose these patients to drug resistance? These questions are unsolved yet!!

Is it possible that there is some malabsorption of the drugs from the GI tract which is leading to poor levels of drug in the systemic circulation.

There is a possibility of genetic predisposition of the bacteria to being resistant to the anti TB drugs by:

- Point Mutation in IFN-g receptor gene located on chromosome 6q
- Absence of IFN-g receptor 1 on cell surfaces
- Functional defect in the up-regulation of TNF-alpha by macrophages in response to IFN-g and poor macrophages killing by macrophages,

CLINICAL PRESENTATION

"catch at first glance"

The clinical presentation in terms of symptoms and signs, of drug susceptible and drug resistant tuberculosis has been comparable all through. In India, any patient with symptoms of fever, cough, hemoptysis, weight loss for more than 2 weeks, with no identifiable cause should be taken as a suspect of pulmonary tuberculosis. Likewise, a patient with extra pulmonary tuberculosis involving pleura, lymph nodes, intestine, genitourinary tract, joint and bones, meninges, brain etc would present with symptoms as per the site involved. A careful comprehensive history at presentation itself should be given a paramount importance as diagnosing TB is important; however, segregating a potential drug resistant patient at first instant has become a necessity now.

Pre-treatment features like history of tuberculosis in past even if treatment was completed adequately, past tubercular disease with poor compliance to treatment or inappropriate treatment prescription, history of treatment failure or defaulter, residence or travel to a location with higher prevalence of drug resistant TB are indicators of possibility of drug resistant TB. Contact with a known or suspected drug resistant TB patient, co-existing HIV infection or Diabetes Mellitus, alcohol intake and smoking are known associations of drug resistant TB.

Table 3: Minimum Mycobacterial count needed for positivity in different diagnostic tools

Diagnostic modality	Minimum number of Mycobacteria needed for positivity (cfu/ml)
AFB smear	10,000-1,00,000
AFB culture	10-100
Gene X Pert	100
Line probe Assay (LPA)	1000-10,000

On-treatment risk features of drug resistance have been poor response to ongoing treatment in a patient in terms of clinical features like persistent symptoms with weight loss, fever, "fall and rise phenomenon" on microscopic examination, inadequate radiological clearance like a persistent cavity and persistent sputum positivity despite appropriate anti tubercular therapy. Although a high index of suspicion is warranted but a clinical worsening should be carefully correlated to rule out IRIS (Immune Reconstitution Response syndrome), while suspecting for drug resistance, which may be challenging at times.

DIAGNOSTIC EVAULATION

"Eyes see, what mind knows"

Drug resistant Tuberculosis being a laboratory diagnosis strictly, diagnostic evaluation holds its importance. An appropriate sample along with an efficient laboratory setting are the key to success. Thus, accredited laboratories have been made mandatory across the country for giving such a vital declaration. Also, it is imperative to understand that a good specimen is equally important for expecting explicit results. For pulmonary tuberculosis, sputum (not saliva) should be sent for evaluation if available. Or else, a bronchoscopic lavage should be obtained. Extrapulmonary tuberculosis (EP TB) gives more challenges to clinicians for specimen collection. Biopsy, fine needle aspirates or tissue should be obtained promptly depending upon the site of involvement. Specimens should be sent for both microbiological (conventional/molecular) and histopathological evaluation with the advent of science and experience, current TB control programme recommends for use of Cartridge based Nucleic acid amplification test (CB NAAT) on first sample itself in patients with extra pulmonary TB, children and People living with HIV.

CONVENTIONAL METHODS

Sputum microscopy

A standard sputum AFB smear (ZN stain) has poor sensitivity of 20-80%, which can be increased with chemical processing (by bleach) with centrifugation and overnight sedimentation by 18% and 23% respectively. Fluorescent technique further enhances the sensitivity by another 10%. LED microscopy poses a similar sensitivity with lesser cost than fluorescent technique. A minimum of 2 sputum specimens should be sent in adequate amount, a spot sample and an early morning sample. If cough is dry in nature or scanty in amount, sputum induction techniques should be tried. However, despite all advantages, it lacks to give any signal for drug resistance. Thus, sputum culture and sensitivity stay a promising resort for diagnosing drug resistance even now.

Sputum culture and drug susceptibility test (DST)

- Culture based DST has been the gold standard diagnostic tool with maximum sensitivity among all the tools available. Firstly, a growth of mycobacteria is sought on either solid or liquid culture media. Following this, species identification is performed by DNA probes, biochemical methods or mass spectrometry.
- Thereafter, growth of mycobacterium complex is observed in drug containing medium and drug free medium to establish the status of drug resistance with the help of concept of "critical concentration." Critical concentration is defined as the level of drug which can inhibit growth of 95% of the wild type of AFB strains, that have not been exposed to the drug but fails to suppress the growth of strains that are resistant to the drug. Importantly, Isoniazid has been tested at two different concentrations. If susceptibility is noted with higher concentrations with resistance at lower concentration, drug can still be used if high serum concentration can be achieved with higher doses.
- Liquid medium requires minimum of a week and solid medium a month duration for DST results. Thus, if conventional method is solely relied upon, an evident delay in appropriate treatment is inevitable. Rapid culture methods with MGIT (Mycobacteria growth indicator tube) and MODS (Microscopic observation of drug resistance) have been introduced using liquid culture media. MODS can be useful in both smear -positive or smear -negative sputa specimen but has been a labor intense technique, thereby limiting its frequent usage. MIC (minimum inhibitory concentration) technique for detecting drug resistance has emerged as a promising future prospect. It assesses drug susceptibility at different concentrations, thus may allow us to use a potent existing drug at a higher susceptible concentrations even if a resistance pattern was found at lower concentrations. We see limited benefits in adding MIC testing as a routine test for DST as giving higher doses of drugs would surely amount to more side effects thereby further reducing the compliance with treatment. However, high dose of isoniazid and moxifloxacin have shown promising results.

Molecular Methods (Table 3)

These are the tests which detect M. tuberculosis complex DNA and most prevalent mutations known to cause resistance. The results are obtained within a few days only. Molecular tests should be done only for diagnosis and not for follow up studies. All molecular tests should be followed by phenotyping cultures.

a. Probe – based assays(Nucleic Acid Amplification Test)

These tests can only detect the presence of mutation in genome but doesn't provide further details. Thus, increases chance of over diagnosing resistance as certain mutations like mis-sense and silent mutation stay silent without amounting to any resistance. Thereby, a false alarm of drug resistance may be raised as all mutations are not deleterious in clinical scenario.

i. X pert MTB / RIF (Gene X pert) identifies resistance to Rifampicin (rpoB) only. Results are supposed to be ready in 2 hours, but the time duration actually depends upon load on laboratories and availability of cartridge. Although the test holds a promising specificity of 98% and sensitivity of 88-93% and holds a promising positive predictive value in both high and low prevalence areas for DRTB. Rifampicin resistance can be detected with an accuracy of 98%. We feel that this assay as compared to conventional methods is less time consuming, less prone to cross contamination, requires lesser bio safety measures and thus should be readily opted for all samples with a good understanding of false positivity.

ii. MTBDRplus (Line probe assay) which detects resistance to isoniazid (katG and inhA gene) and rifampicin (rpoB gene) both. This assay requires only 1000 to 10,000 cfu/ml for detection of mycobacteria. Results are obtained in 1-2 days. It has been validated only for sputum positive samples only as yet, with a clear advantage of providing drug susceptibility status in as early as few hours. On smear-positive sputum specimens, LPAs hold a high sensitivity (≥97%) and specificity (≥99%) for the detection of RIF resistance alone or in combination with INH (sensitivity ≥90%; specificity ≥99%), on isolates of M. tuberculosis. It can detect high level and low level resistance to Isoniazid thereby giving a chance to use high dose of Isoniazid, if low level resistance is noted. Overall accuracy for detection of MDR-TB has been 99%. MTBDRs/ is a newer line probe assay detecting resistance to injectable agents(rrs gene) and fluoroquinolones(gyrA gene) as well, however poor sensitivity dropping as low as 30% has limited its use as yet.

b. Sequence-based assays

These are more advanced tests under pipeline for now, wherein, not only a gene mutation but also the whole sequence can be detected thereby giving more specific information regarding possible drug resistance. They can detect resistance not only to isoniazid and rifampicin, but also to fluoroquinolones and injectable agents. Pyro sequencing (detects pyrophosphate released from DNA synthesis), Sanger sequencing, next generation sequencing are the known sequence based assays still under investigation and not approved by FDA.

TREATMENT

"An expert's call"

As drug resistant tuberculosis is taking a toll worldwide, all the elements of effective therapy should be well understood by patient and physician both. The survival rates of drug resistant tuberculosis have been less than that of drug susceptible tuberculosis. However, our experience with "unpublished data" has been showing a gratifying success rate of approximately 70-75%, in patients with MDR TB. All it needs is care, dedication and caution at each step from suspecting till cure. Unfortunately, relapse rates have been high but can be tackled with early suspicion, prompt diagnosis, apt regimen, compliance to treatment and with surgical intervention in carefully selected individuals, where disease is well localized like a persistent cavity despite microbiological clearing.

CHEMOTHERPY: In current era, time has come to give up the concept of "one in all" where a pre-determined regime was administered to all the patients and rather embrace "all in one" principle where an individualized regimen should be prescribed depending upon clinical background and resistance pattern and all what should be done is ensured in every single patient fighting with DR TB.

Basic principle of treatment regimen:

1. Notification of all resistant cases should be done.
2. In patients with MDR TB, possibility of pre XDR TB or XDR TB should always be ruled out.
3. Regimen for drug resistant TB should be made with knowledge of previous exposure to drugs, details of previous treatment, known co-morbidities and potential drug interactions.
4. Individualized therapy is better in areas with high prevalence of DR TB (MDR TB, Pre- XDR TB and XDR TB) especially in metro cities like Mumbai.
5. Daily regimen with doses as per weight, throughout the course.
6. For an MDR TB regimen at least 5 to 6 effective new drugs should be added, with one injectable class II drug, to be used for 6-8 months for at least 5 days a week.
7. Cycloserine or terizidone form an integral part of DRTB regimen despite no available DST for them.
8. Another rifamycin derivative called Rifabutin can be used in certain situations like TB- HIV co infection where a protease inhibitor is used in regime and in those cases where low resistance to rifampicin is seen.
9. Clinical improvement and radiological correlation is a must to understand the responsiveness.
10. Serial monitoring with AFB smear and cultures

Table 4: WHO recommendations for Treatment in DRTB

Drug Resistance	Regimen in Intensive phase	Regimen in continuation phase
ISONIAZID (H)	(3-6) Km Lfx R E Z	(6)Lfx R E Z
RIFAMPICIN (R)	(6-9) Km Lfx Eto CsZ E H	(18) Lfx Eto Cs E H
HR (MDR TB)	(6-9) Km Lfx Eto Cs Z E	(18) Lfx Eto Cs E
HR + FQ + INJ. AGENT (XDR TB)	(6-12) Cm PAS Mfx High dose H Cfz Lzd Amx/clv	(18) PAS Mfx High dose H Cfz Lzd Amx/clv

should be done religiously, with a close look out on early worsening.

11. In clinical practice, absorption studies (for rifampicin and isoniazid) should be asked in a selected subgroup for those patients who show persistent poor outcome on chemotherapy despite sensitivity to rifampicin and isoniazid.

12. Whenever required a surgical intervention at right time is worthwhile, not only to curtail the infection but also to reduce relapse rates. Post operatively, duration of therapy stays the same, amounting to 2 to 3 years depending upon the individual's clinical status and resistance pattern.

- If MDR isolate shows additional resistance to other drugs as well (Table 4).

a. If resistance to both E and / or Z is seen, omit them both from MDR regimen, instead add PAS to be continued throughout IP (Intensive Phase) and CP (Continuous Phase)

b. If resistance either of FQ (Mfx/Ofx) is noted, add the remaining susceptible FQ like gatifloxacin or high dose Mfx to MDR regimen.

c. If resistance to all the FQ is seen, omit Fq from MDR regimen, instead add PAS+ Cfz+Lzd to MDR regimen throughout and extend IP from 6-12 months.

d. If resistance to either of injectable SLD (Km/Cm/Am) is seen, use the remaining susceptible injectable agent in the MDR regimen.

e. If resistance to all the injectable SLD is seen, omit them from MDR regimen and instead add Cfz+PAS+Lzd and include group V drugs in the regimen

SURGERY IN RESISTANT TB

Surgical option has again captured its importance as was in pre-treatment era. It has been strongly recommended in MDR TB, pre- XDR TB, XDR TB with a localized extensive disease. Candidates with positive cultures in 4 to 6 months of ATT, persistent cavity in lung despite smear or culture conversion, complications like intractable haemoptysis or persistent broncho-pleural fistula should be readily evaluated for surgical option with a careful pre-operative assessment. All surgical candidates should be primed with ATT prior to surgery preferably till sputum conversion and should be continued on the same regime till the stipulated time duration, as advised. Surgical excision of maximal possible diseased area should be aimed at, so as to ameliorate relapse rates

WHAT'S NEW

"Innovations are a must, to see a different future"

- New chemotherapy drugs in DR TB, which would be shortly available

Bedaquiline

A diarylquinoline that targets mycobacterial ATP synthase enzyme thereby limiting the energy replenishment to MTB in body. It has shown a strong bactericidal and sterilizing action. RNTCP has made this drug available at 6 centers for now, as an add- on drug to ongoing background regimen and not as a replacement. It has been approved in patients aged more than 18 years with pulmonary MDR TB. Non-pregnant females on non-hormonal birth control measures, willing to continue the same throughout the therapy or have entered in their menopause for at least 2 years can be enrolled for the therapy. If patient has stable arrhythmia, then a cardiac clearance is mandatory prior to therapy. Recommended oral dose is 400mg once daily for 2 weeks, followed by 200mg 3 times a week for 22 weeks with food. Indoor monitoring for initial 2 weeks on Bedaquiline is preferred as QTc prolongation is a known side effect. Thus, ECG monitoring should be done and also drugs prolonging QTc interval should be best avoided while patient is on Bedaquiline

Delaminid

Is a nitro-dihydo-imidazooxazole derivative which inhibits the mycolic acid synthesis. It is known to cause sputum conversion in 2 months. Recommended dose is 100 mg twice daily orally for 6 months added to an ongoing regimen. Similar side effect is noted with QTc interval prolongation. Thus, patients should be monitored for the same.

- Bangladesh short treatment course regime in MDR TB/ RRTB (Rifampicin Resistant TB) cases has gained a lot of attention with WHO also envisaging the same in 2016 update. A 9-12 month short term treatment can be offered to a selected subgroup of patients, who suffer from Pulmonary TB with no exposure to any of the second line agents in past. Children and pregnant women are excluded. However, with such a surge in resistance in our nation, we suggest refraining from taking such a huge risk of limiting the duration, as it would rather cause more harm than offering any benefits.

PREVENTION

"let it not touch you"

- Diagnosed TB patients should complete full course of anti-TB treatment at first go only.
- During the intensive phase, patients should ensure contact precautions, as much as possible.
- Pace of diagnostic evaluation should be escalated even more to catch the danger at its earliest.
- Minor side effects should be dealt with will power and symptomatic measures to ensure the compliance
- Every patient with TB should be suspected for drug resistance from first visit only, in our country.
- Close follow up of patients is a must.
- No single drug should be added to a failing drug regime, if resistance to drugs is suspected.
- Proper treatment of MDR –TB first time is the best way to prevent XDR –TB.
- Over all miss use of fluoroquinolones should be stopped in our country.

An effective drug regimen should be preferably devised by an expert only, as a previous ineffective regimen has been established as the most eminent risk factor for development of drug resistance eventually. We strongly endorse to a better understanding of current TB scenario by treating physicians, patients and government. Prompt initiation of an appropriate regime, in adequate doses for the recommended duration are all important links towards a vision of TB free India.

REFERENCES

1. Extensively drug-resistant tuberculosis in India: A review Joy Sarojini Michael & T. Jacob John Department of Microbiology, Christian Medical College, Vellore, India. *Indian J Med Res* 2012; 136:599-604.
2. World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis: 2016 update. WHO, Geneva 2016
3. Totally drug-resistant tuberculosis in India. Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C. *Clin Infect Dis* 2012; 54:579.
4. Centers for Disease Control and Prevention (CDC). Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs--worldwide, 2000-2004. *MMWR Morb Mortal Wkly Rep* 2006; 55:301.
5. Palomino JC. Newer diagnostics for tuberculosis and multi-drug resistant tuberculosis. *Curr Opin Pulm Med* 2006; 12:172.
6. World Health Organization. Global tuberculosis report 2015. WHO, Geneva 2015
7. Comparison of Xpert MTB/RIF with Line Probe Assay for Detection of Rifampin-Monoresistant *Mycobacterium tuberculosis* Syed Beenish Rufai etal. *J Clin Microbiol* 2014; 52:1846–1852.