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An update on pathogenesis and management of tuberculosis with special reference to drug resistance

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ABSTRACT

Drug resistance in tuberculosis (TB) is a global problem and both developed as well as under developed parts of the world are predisposed to drug resistant TB. Multiple drug resistant-TB (MDR-TB) designates the very strain of the pathogen which is resistant to at least two primary anti-TB drugs isoniazid and rifampicin. This strain after acquiring a further bacillary resistance to any second line injectable drug and any of the fluoroquinolones is termed as extensively drug resistant TB (XDR-TB). The present review is endeavored to recapitulate the contemporary state of multidrug resistance in TB, the pathophysiology and recent developments for a rapid and reliable detection of the infection and management of MDR-TB. The challenge of MDR-TB management must be embarked on by skilled doctors at operational BCL-3 laboratory facilities where all allied services for the in-vitro sensitivity testing of mycobacteria are available because it includes extended treatment with costly second-line drugs containing meticulous toxicity. Even more dreaded are some newly emerging TB strains namely XDR-TB which is resistant to many more anti-TB agents (such as isonicotinic acid hydrazide and rifampicin plus second line injectable streptomycin, amikacin and kanamycin). Newer discovery of novel anti-TB drugs through recent research regarding the management of drug resistant tuberculosis would help avert and eradicate MDR-TB as well as XDR-TB. For shortening of the TB-treatment regimen, a few drugs, especially gatifloxacin and moxifloxacin, are being tested, while PA-824, OPC-67683 and TMC-207 are also being studied for both drug resistant and drug susceptible disease. Given the past global trends in MDR-TB, if aggressive preventive and management strategies are not implemented against it, XDR-TB would emerge to a larger extent which would severely cripple global control efforts of TB. However, very recently a newly discovered drug bedoquinoline is demonstrating strong promise towards containment of XDR-TB.

1. Introduction

Tuberculosis (TB) is one of the leading causes of infectious deaths and the increasing rates of drug-resistant TB are of global concern[1,2]. World Health Organization (WHO) declared, an estimated 650000 prevalent cases of multidrug-resistant tuberculosis (MDR-TB) occurred globally in 2010[3-5]. Geographically, the burden of TB is highest in Asia and Africa[6]. In the global tuberculosis report of the year 2006, WHO informed that, two billion people (one third of the human population) had been estimated to have latent infection with *Mycobacterium tuberculosis* (MTB). 8.8 million new cases, nearly two million deaths and 450000 new

MDR-TB cases are estimated to occur every year. Roughly 200 million people are estimated to acquire active disease progression from latent TB infection during the whole lifetime[7]. Despite the Millennium Development Goal of WHO, targeting to halt and reverse the TB epidemic by 2015 has not been achieved; the global burden of TB remains enormous till date. In 2011, there were an estimated 8.7 million new cases of TB (13% co-infected with HIV) and 1.4 million people died from TB, including almost 1 million deaths among HIV-negative individuals and 430000 among people who were HIV-positive[6]. Over 70% of TB patients are in the economically productive age of 15 to 45 years[8]. Developing countries account for 95% of TB cases and 98% of deaths related to the disease. Of these all, the maximum number of instances were accounted by population of the Asia (59%) and the Africa (26%)[7]. Globally, 3.7% of fresh instances along with 20% of formerly treated cases have been projected to have MDR-TB[4-6]. About 450000 people developed MDR-TB in the world in 2012. More than half of these cases were in India, China and the Russian

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Federation[9]. According to WHO, if proper control measures are not instituted, it is estimated that between 2002 and 2020, approximately one billion people will be newly infected, over 150 million people will get sick, and 70 million will die of TB. The prevalence of primary and acquired multidrug resistance in India was reported as 3.4% and 25% respectively in 2005[10]. HIV is the most powerful risk factor for the conversion of TB infection to active disease and TB is the most common opportunistic infection in HIV positive patients. In the past few years, genetic and molecular insights have unraveled the mechanisms involved in the acquisition of drug resistance by MTB and have also assisted in the development of various molecular strategies to rapidly detect MDR-TB[11]. Our ultimate goal should be to do away with the deadly disease by 2050 *i.e.*, reducing the rate of incidence of infection below one in a million[6].

2. Pathophysiology and immunology of drug susceptible MTB and MDR-TB

2.1. Route and site of infection

The tubercle bacilli move into the host body by means of the breathing route and preferentially reside in the oxygen rich lung tissue because the intracellular pathogen is an obligate aerobe. From the place of initial infection the pathogen then spreads to its favored sites like the apex of lung and thereafter the regional lymph nodes and to other parts of the body through the lymphatics or blood[12]. Reviewers from India also recounted about the other routes of TB infection *e.g.*, ingestion of infected sputum, or direct spread from infected contiguous lymph nodes and fallopian tubes in immunocompromised or HIV infected patients, as depicted in the Figure 1[13].

2.2. Host-pathogen interaction

The initial affair concerning the interaction between host's defence mechanism and the pathogen that decide on the fate of the infection is the phagocytosis of MTB by alveolar macrophages. Following that, with an influx of activated lymphocytes and macrophages, a granuloma is formed at the site of lesion. This creates a microenvironment that averts the spread of the mycobacteria[14]. This microenvironment can lead to death of the macrophages thus yielding necrosis at the center of the lesion[15]. Lesions in persons with an ample immune system, successfully controls the infection but the ones with compromised immune system fall prey to the primary progressive tuberculosis[15-17]. Because, in case of less immunocompetent persons, even after the initiation of granuloma, it eventually fails to capture the bacilli. The exponential growth is checked when the bacilli become contained in the caseous (formed by accumulation of dead macrophages) centres of granuloma[12]. The mycobacteria still carry on proliferating self in every 25-32 h even after it has been gulped by the macrophages[16]. A cell mediated immune response involves the macrophages, T-lymphocytes and related other signaling molecules when induced by exposure of a healthy individual to TB[18]. TB infection, in the presence of a weakened immune system, spreads mercilessly leading to the development of active disease[19,20]. Figure 2 offers a diagrammatic

view of the host-pathogen interaction in cases of immuno-competent and immuno-compromised individuals.

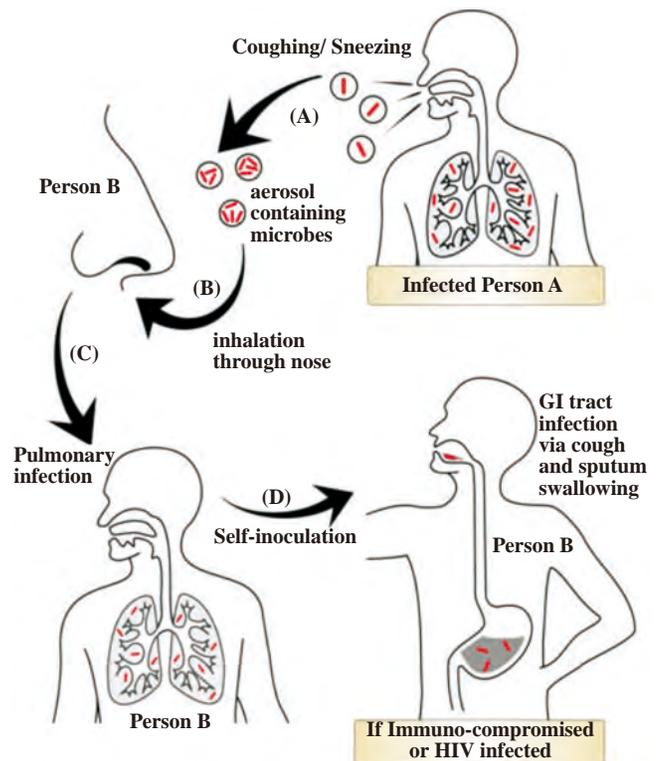


Figure 1. The overall picture of the common modes of infection caused by *Mycobacterium tuberculosis*.

A: The bacterium is spread by an infected individual by coughing or sneezing, when the pathogen comes out of his (Person A) body and starts residing in the form of aerosol; B: The aerosols, containing MTB, are inhaled by a healthy person (Person B) with air; C: The next step is often a pulmonary infection when the pathogen reaches the effective titre in the lung of person B; D: Self-inoculation results in the spread of infection from the lung to GI tract. 'Self-inoculation' is used because it takes place when person B, infected with pulmonary tuberculosis, swallows his own infected sputum.

2.3. Immunity against the pathogen

Phagocytosis is the first event to take place in the case of a primary infection. Other components of innate immunity against TB include the subsequent secretion of interleukin (IL)-12, and Natural resistance associated macrophage protein (Nramp), and the activation of neutrophils and natural killer cells. Raja A, in 2004, proposed that the predisposition to MTB infection may not be due to the role of *Nramp1* gene[12]. Neutrophils are the first cells to reach the site of multiplication of bacilli. Natural killer cells, γ/δ and α/β cells are the next to follow the neutrophils. The white blood cells specifically neutrophils can be enhanced by the granulocyte macrophage colony stimulating factor towards the phagocytosis of the pathogen[21]. Human neutrophil peptides (HNPs) 1, 2 and 3 are involved in innate host defense and acquired immune response, which is possibly also associated with the genesis of multidrug-resistant tuberculosis[22]. Zhu *et al.* demonstrated the low concentration of HNPs (HNP1-3) in plasma to be linked with incidents of MDR-TB development. A significant reduction in natural killer cell activity has also been involved in the emergence of MDR strains of tuberculosis[22].

Various epidemiologic studies and experimental laboratory investigations provided data in support of the danger of drug-resistant strains of TB as this review entailed so far. So, it can be assumed that the decade-old reason of numerous epidemics have become more challenging over time with the emergence of drug-resistant strains and extremely difficult to contain when it evolved to MDR-TB as well as to the extensively drug resistant TB (XDR-TB).

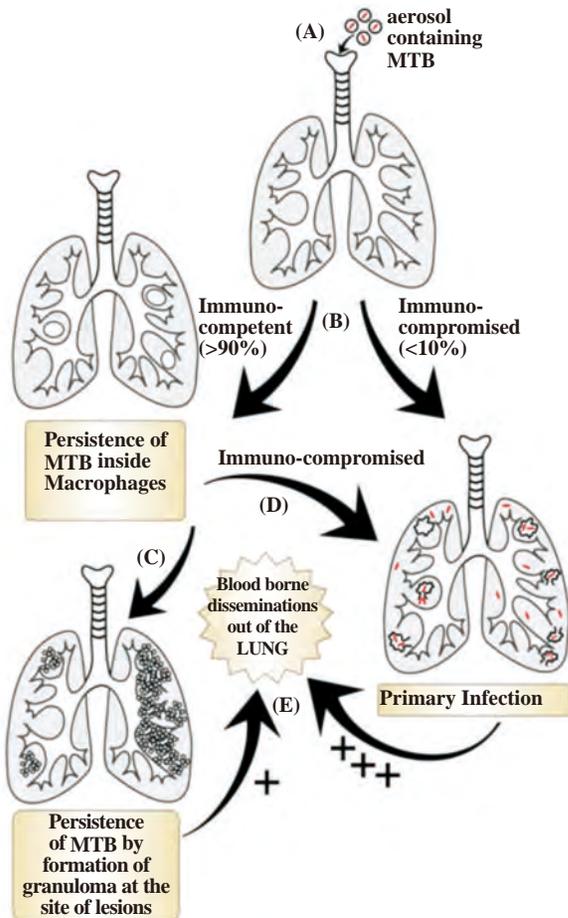


Figure 2. The pathogenicity of the bacterium in a population, and the spread of infection from one organ to another.

A: The first step in the pulmonary infection is the entry of the bacterium through the trachea, in the form of aerosol; B: Depending on the immune-competence of the victim, the pathogen may reside inside the alveolar macrophages without showing any symptom or can cause severe pulmonary infection; C: Often at a later stage the macrophages lyse to free the pathogen, in the asymptomatic lung, which forms granuloma at the site of lesion and can persist there for a long period of time; D: A carrier of TB with asymptomatic lung may however develop primary pulmonary infection, if at any time the individual becomes immuno-compromised; E: The contagion gains access to the other organs through blood borne dissemination. This spread of infection happens to be more with immuno-compromised infected individuals. However, the dissemination is also possible, with much less frequency from the lung of a immuno-competent individual.

2.4. Drug-resistant tuberculosis

TB strains, singularly resistant to individual anti-tuberculosis drugs, have been reported since the late 1950s. Be there or not any bit of resistance developed against any other drugs than isoniazid (INH) and rifampicin (RIF) the strain is a multidrug resistant one if it is resistant at least to the two key anti-TB drugs mentioned[10]. Bacterial isolates that are multiple-resistant to any other combination

of anti-TB drugs other than INH and RIF are not classified as MDR-TB. INH, the most widely used tuberculosis drug available, ensures early sputum conversion (sputum positive to sputum negative by Ziehl-Neelsen method) and helps in decreasing the transmission of TB whereas RIF, by its mycobactericidal and sterilizing activities are crucial for preventing relapses. Thus, INH and RIF are key medications in the treatment of TB. Patients, sometimes, are forced to take such toxic drugs for prolonged time. So, to imply an appropriate remedial intervention the *in-vitro* sensitivity testing will be needed for an effective discrimination between MDR-TB and a common drug-resistant strain.

XDR-TB is said to be the MDR-TB that acquired resistance to fluoroquinolones like ciprofloxacin or ofloxacin or levofloxacin and also to any one of the second-line injectable drugs like kanamycin or capreomycin, or amikacin[10,23]. Some contend that XDR-TB strains have emerged from the mismanagement of MDR-TB and once created can spread from one person to another (Figures 3 and 4). The main beliefs regarding the therapy for both MDR-TB and XDR-TB are very similar. The only dissimilarity between the two forms of the disease remains in the rate of mortality. In XDR-TB it is much higher than that in MDR-TB, because effective options of therapy are scarce in XDR-TB compared to MDR-TB[24]. The true scale of XDR-TB is unknown as many countries lack the necessary equipment and capacity to accurately diagnose it.

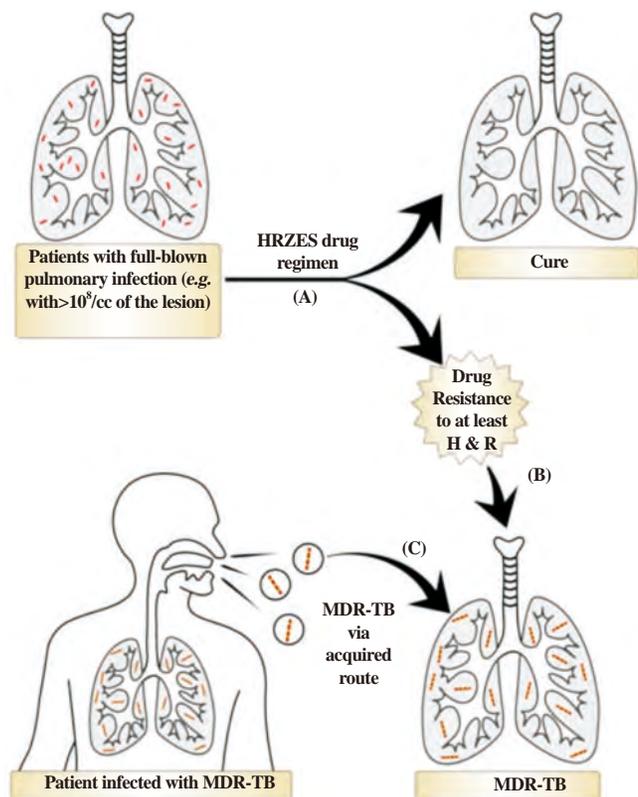


Figure 3. The figure shows the course to the formation of MDR-TB.

A: When the pathogen attains the titre of 10^8 /cc in the lung, a pulmonary infection takes place and the infected person undergoes a treatment regimen, including the first line drugs INH (H), RIF (R), PZA (Z), ETH (E) and STR (S), which continues for at least 6 months; B: Out of the treated population, the fraction of the patients who are not cured carries the group of pathogen which gets resistance to at least INH (H) and RIF (R). The fraction thus gets infected by MDR-TB; C: There is another group of MDR-TB patients who, as healthy persons, develop the infection by direct inhalation of pathogen containing aerosols from another MDR-TB patient.

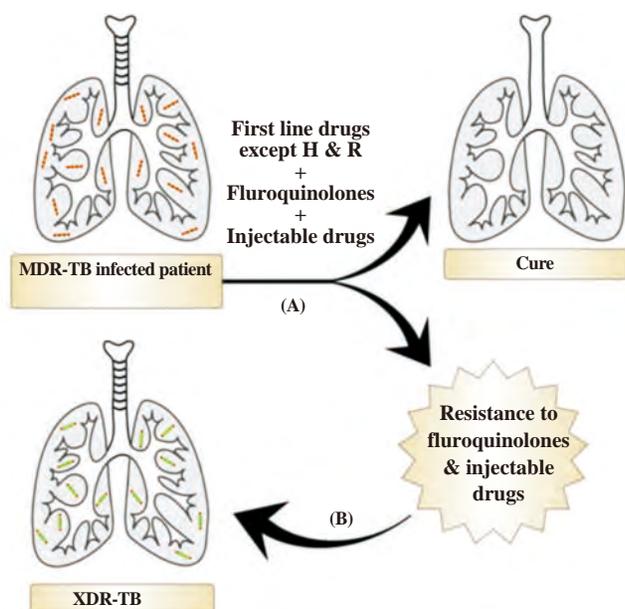


Figure 4. The formation of XDR-TB from MDR-TB.

A: The MDR-TB patients undergo a treatment regimen including all the first line anti-TB drugs except INH (H) and RIF (R), fluoroquinolones and injectable drugs like kanamycin etc; B: A segment of the MDR-TB infected population, due to improper medication and care, develops XDR-TB when the pathogen acquires resistance to the fluoroquinolones and injectable drugs.

2.4.1. Types of drug resistance in tuberculosis

There are two types of drug resistances-Primary and Acquired. Primary drug resistance is said to be the one of the kinds when it takes place in a patient before the individual had ever received any anti-TB drugs while the acquired one is the other kind which may be characterized in a patient who has already taken anti-TB therapy. When one is not sure whether the resistance is primary or acquired due to concealed history of previous treatment or unawareness of treatment taken before, it is known as primary or initial drug resistance. Combined resistance is defined as sums of primary and acquired resistance[10].

2.4.2. Epidemiology of drug-resistant TB

MDR-TB and XDR-TB have caused global epidemics. Around 650 000 people were estimated to have MDR persistence out of a total diseased population of 12 million in 2010, and 9% out of the MDR infected population had XDR-TB globally[1]. MDR-TB commonly develops in the course of TB treatment[25] (Figure 3). Such consequences result when the patients are either reluctant to take doses correctly or fail to complete the whole treatment or the clinician prescribing a wrong treatment profile. MDR-TB strains look as if they are not as much of efficient to be infectious. People who have weakened immune systems (e.g. patients with HIV) are more susceptible to catching TB[26-28]. The mortality rate of MDR-TB is as high as that of lung cancer. In a survey of 35 countries in 1997, the highest-found rates for developing MDR-TB were in the former USSR, the Baltic States, Argentina, India and China. Reliable data are inadequate regarding the epidemiology of MDR-TB in India[29]. In the third world countries, including India, the common drug resistant strains have been a threat for quite some times now. But both INH and RIF resistant strains are creating perilous consequences in recent times[30,31]. This is alarming to cite our observation that out

of 1029 TB-positive patients (new & previously treated) screened in just one tuberculosis unit (TU) in West Bengal, India, during January 2011 to December 2012, 21 MDR-TB isolates (our unpublished data) were detected.

In recent years, XDR-TB has been reported from many countries to emerge from MDR-TB by the mechanism described earlier (Figure 4). More than 75 countries have reported to have faced at least one XDR-TB threat by the end of 2011[32]. The epidemiology of XDR-TB has yet not been well studied, but it is believed that XDR-TB does not transmit easily in healthy populations, but is capable of causing epidemics in populations which are already stricken by HIV and therefore more susceptible to TB infection. During the first ever outbreak of XDR-TB which took place in Kwa Zulu-Natal of South Africa in 2005 more than 98% of all victims died within 25 days of infection[33].

In 2010, a total of 290000 cases were reported to have pulmonary MDR-TB worldwide, out of which 44% cases were accounted by India and China. Among new cases of infection the MDR-TB was observed to have acquired a population of 3.4% whereas it was noticed to be around 21% in case of retreatment[32]. The WHO has examined the extent of the problem of MDR-TB in cross-sectional surveys of drug resistance in either clinical series or whole-country cohorts. Cross-sectional surveys almost certainly underestimate the burden and number of cases of MDR-TB because they do not take into account the numerical burden of TB in the highly affected countries.

3. History of drug development against tuberculosis

The pathogen responsible for tuberculosis, known from primitive times came to news only when Robert Koch discovered MTB in 1882. However, no appropriate therapeutic intervention was on hand to control TB, before the 1940s. The first specific anti-tuberculosis drug, streptomycin (STR), was reported from the USA during 1944 and clinically used soon after[34]. STR, an injectable agent, is used especially for children with tuberculous meningitis, but many children relapse after a few months of treatment because of the development of STR resistant bacteria. A European group of scientists developed para-aminosalicylic acid (PAS) for tuberculosis treatment and it was used in the late 1940s[34]. The optimal length of treatment for pulmonary tuberculosis using a combination of STR and PAS is two years. The sub-optimal use of STR and PAS has contributed to the emergence of drug resistant TB.

Subsequently, INH was clinically used for the first time in 1952[34] and it quickly became the most popular anti-TB drug especially due to its low cost. The treatment length could be reduced from 24 months to 18 months by using a combination of STR, PAS and INH. Other anti-tuberculosis drugs, pyrazinamide (PZA), Ethambutol (EMB), Ethionamide (ETH) and cycloserine (CS) were discovered later and added to the treatment regimen. PZA caused unacceptable side effects in its effective dosage. EMB alone was not very efficient in killing bacteria. It is useful in combination with other drugs for preventing the emergence of resistance. ETH and CS are both hampered by poor bacterial killing ability and troublesome side effects. The most potent TB-drug, RIF, was discovered in the late 1960s[34]. The length of tuberculosis treatment could be reduced

to as little as six months with a combination of INH, RIF, PZA, and EMB. The currently used therapy regimen in India, according to the Revised National Tuberculosis Control Programme (RNTCP), is 2 months with all four antibiotics (INH + RIF + PZA + EMB) followed by another four months with INH and RIF[35].

4. Molecular mechanisms of drug resistance in tuberculosis

Most of the molecular mechanisms of acquiring resistance to drugs have been described in the very recent past and by now people have started to realize the truth behind the development of drug resistant strains of MTB. Accumulations of mutations in individual drug target genes are involved in generation of MDR-TB (Table 1). For nominal anti-TB drugs, the chance of developing resistance is considerably higher while the bacillary load is directly responsible for the equivalently increased rate of mutation. A tuberculosis cavity usually contains 10^7 - 10^9 bacilli/cc. While INH resistance takes place at the rate of 10^{-6} replications in a pathogen and the rate becomes even rarer in case of RIF resistance accounting for only 10^{-8} replications, the likelihood of occurring both incidents spontaneously is simply 10^{-14} applying the multiplicity rule. Therefore the emergence of simultaneous binary resistance to RIF and INH are very rare because MTB cannot be found in such a large number even in extensive cavitary pulmonary TB. The principal means to cause MDR-TB is owing to the deviation in the particular target genes for specific drugs[36,37] and sum total accumulation of single resistance to individual anti-TB drugs.

Table 1

The Gene(s) involved in anti-tuberculosis drug resistance.

Drug	Gene
RIF	<i>rpoB</i> (RNA polymerase subunit B)
INH	<i>katG</i> (Catalase-peroxidase) <i>oxyR</i> (Oxidative stress regulator) <i>ahpC</i> (Alkyl hydroperoxide reductase)
INH-ETH	<i>inhA</i> (Enoyl acyl carrier protein reductase)
Streptomycin	<i>rpsL</i> (Ribosomal protein subunit 12) <i>rrs</i> (16 ribosomal RNA)
Fluoroquinolone	<i>gyrA</i> (DNA gyrase A)
PZA	<i>pncA</i> (Pyrazinamidase)
EMB	<i>embCAB</i> (Arbinosyl transferase)

4.1. Intrinsic mechanism of resistance

It has long been well-known that the intrinsic mechanism of drug resistance of MTB is accredited to the odd cell wall construction which confers on the pathogen a scanty penetrability of the chemotherapeutic agents as well as several antibiotics owing to the presence of very long fatty acid chains (mycolic acid) in the cell wall[38]. Other than this, the efflux mechanism is a vital element in the natural resistance to various class of anti-TB agents including fluoroquinolones, aminoglycosides and tetracyclins, playing a crucial role in causing XDR-TB[39].

Moreover, porins are believed to play an important role in drug resistance, when mutated. A study showed that a few hydrophilic fluoroquinolones like norfloxacin, and even Chloramphenicol, makes entry into the bacteria through specialized protein channels

like porins in its surface[40]. The β -lactam antibiotics mainly exploit the same mechanism of diffusion entry but fail to inhibit because of the presence of β -lactamase enzyme in MTB[41]. The enzyme activity is encoded by *blaC* and *blaS*. Recent studies have shown that a mutation in a protein Rv1698 resulted in an increased minimal inhibitory concentration (MIC) of hydrophilic bacterio-static or bacterio-cidal compounds[42]. A concurrent bioinformatics study has identified the protein Rv1698 and another protein Rv1973 to be the outer membrane proteins that are very likely to have roles in the intrinsic resistance machinery[43]. In addition to the permeability barriers and β -lactamase activity induced intrinsic drug resistance; the antibiotic tolerance may also be achieved by the body's own biological adaptations[44].

4.2. Acquired mechanism of resistance

Acquired drug resistance is a consequence of spontaneous mutations in chromosomal genes, producing the selection of resistant strains during sub-optimal drug therapy[45]. After analyzing the route map of developing drug resistance through molecular and genetic analysis it is realized that the pathogen acquires resistance in either of the two main mechanisms. One leading to excessive production of the drug target and thus titration of the applied drug while the other leads to change in the targeted molecule by means of mutation[46,47].

As mentioned earlier, when a specific strain of TB develops resistance to INH and RIF, it is termed as multidrug resistant whereas an XDR strain is generated when a MDR-TB strain becomes resistant to fluoroquinolones and one or more injectable drugs like kanamycin, amikacin, or capreomycin.

Because spontaneous mutations causing resistance to two or more drugs simultaneously, is extremely unlikely a probable multifaceted link amid conventional mutations linked with resistance to a single drug could lead by a stepwise mechanism to resistance to other drugs. It is for the reason that an MDR or XDR phenotype in MTB has not been found to be caused by any single pleiotropic mutation[48].

4.3. Mechanisms resulting in drug resistant tuberculosis

Resistance to the most important first line anti-TB drug, INH, can be mediated by several genetic mutations involving *ndh*, *kasA*, *inhA*, *ahcP* and *katG*. INH is a pro-drug that requires activation by the catalase/peroxidase enzyme encoded by *katG*[49]. Banerjee *et al.* and others reported that *katG*-activated INH inhibits *inhA* (the target for INH), the NADH-dependent enoyl-acyl carrier protein reductase, to interfere with mycolic acid (indispensable element of its cell wall) synthesis[50]. Therefore, any mutation that reduces the activity of either *inhA* or *katG*, will result in resistance to INH. The most common mutations of *inhA* is hugely targeted in the promoter region. That mutation in the *inhA* gene can cause resistance to a second line drug, ETH alongside INH was shown earlier. It was probably due to the structural similarity of ETH with INH[51]. Moreover, Banerjee *et al.* and others observed that INH resistance not only maps in the *inhA* open reading frame but also in the *mabA*, gene which is the proximal part of the *mabA-inhA* operon[52].

When the β -subunit of the RNA polymerase is mutated, it hinders the elongation of the mRNA priorly interfering with the binding of

RIF thus resulting in RIF resistant strain generation[53]. This drug counteracts to the aggressively multiplying pathogen which are at the same time very slowly metabolizing bacteria[54]. The resistance is produced by the conformational changes, a downstream event to the mutation of *rpoB*, which determine a low affinity for the drug resulting in resistance[55]. The mutations, generating the resistance to RIF, have been detected to span a 'hot-spot' region of 81 base pairs (bp) on *rpoB*. Termed as RIF resistance determining region is the length in the nucleotide sequence that covers codons 507-533[56]. Although some studies did report to have shown mutations outside of the hot-spot, RIF resistance determining region is responsible for about 96% of RIF-resistant MTB isolates. An important finding regarding the RIF-resistant strains is that all strains in this category also show INH-resistance. This led to propose the RIF-resistance detection as a surrogate molecular marker for MDR[57].

5. Management of MDR-TB

MDR-TB is a serious problem and is very difficult to treat. MDR and XDR tuberculosis is generally thought to have high mortality rates primarily because of the limited treatment options and poor prognosis. By prolonged treatments of second line drugs, MDR-TB can be cured but these are more expensive than first line drugs and have more adverse effects[58].

The mortality rate of MDR-TB is up to 80%, which depends on a number of factors, as given below: how many drugs the patient is resistant to; how many drugs the patient is given; or not the patient is given an injectable drug; the expertise and experience of the physician; how co-operative the patient is with treatment; and whether or not the patient is HIV positive.

For efficient management of problematic MDR-TB or XDR-TB, the pivotal attitude should be to prevent its emergence. Inadequate adherence or complete non-adherence to recommended treatment was soon recognized as a behavioral problem to cause MDR-TB by achieving acquired resistance due to treatment failure[59].

To overcome this problem, the directly observed therapy was made known to us for the first time as an ambulatory treatment supervision in 1960s. Later, WHO recommended a short course combination chemotherapy with the first line anti-TB drugs to emphasize directly observed therapy, thus introducing directly observed therapy short course (DOTS) in 1993. In short, DOTS implies that the TB infected person swallows short course anti-TB drugs under direct observation of a health worker or other trained individual. The entire course involves two phases: intensive phase and continuation phase.

However, DOTS has been inadequate for the treatment of MDR-TB patients with first line drugs. Earlier studies have already reported that most frequent cases of MDR-TB are observed in the patients with treatment failure by the first line drugs[60]. Hence, the necessity to tackle MDR-TB introduced the DOTS-plus strategy with second line drugs in 1998. The five elements of the DOTS plus strategies are as follows[61]: 1) An individualized treatment is better suited instead of a standardized one; 2) Facility for providing culture facility along with that for susceptibility testing; 3) Sufficient supply of different types of costly second line drugs; 4) Operational studies are necessary for evaluation of the effect of this shift; and 5) Technical and financial support from international organizations & local

governments.

DOTS-plus unquestionably aids towards the improvement in outcomes of MDR-TB and therefore it should certainly be included as an essential part in the MDR-TB treatment[62]. Response to MDR-TB treatment is monitored by culturing of sputum repeatedly. The treatment must be continued for a least possible period of eighteen months. It could only then be stopped when the sputum test has given negative results for at least nine months consistently. Persons with drug resistant TB are to be accommodated in rooms with negative-air pressure and the ward should never contain any immunocompromised patient and anybody who enters inside the infected space, should be available with suitable masks comprising high efficacy particulate air filters (N-95) outside the entrance[62]. All these are done to prevent the spread of MDR-TB.

However, the overall scenario threatens with risks of under-diagnosed MDR-TB and the inadequacy in its treatment. While around 2% of the newly infected patients and 6% of the previously treated patients accounted to have MDR-TB; among them the population who enrolled for treatment, accounted for only 16% of all the MDR-TB patients worldwide in 2010[1]. Targeting exclusively the high risk patients in monitoring and combating MDR-TB may not help in the global efforts because doing so would lead to failure towards targeting a considerable share of MDR-TB cases[63]. A multidimensional methodology for the management of challenging outbreaks of MDR-TB has been illustrated in Figure 5.

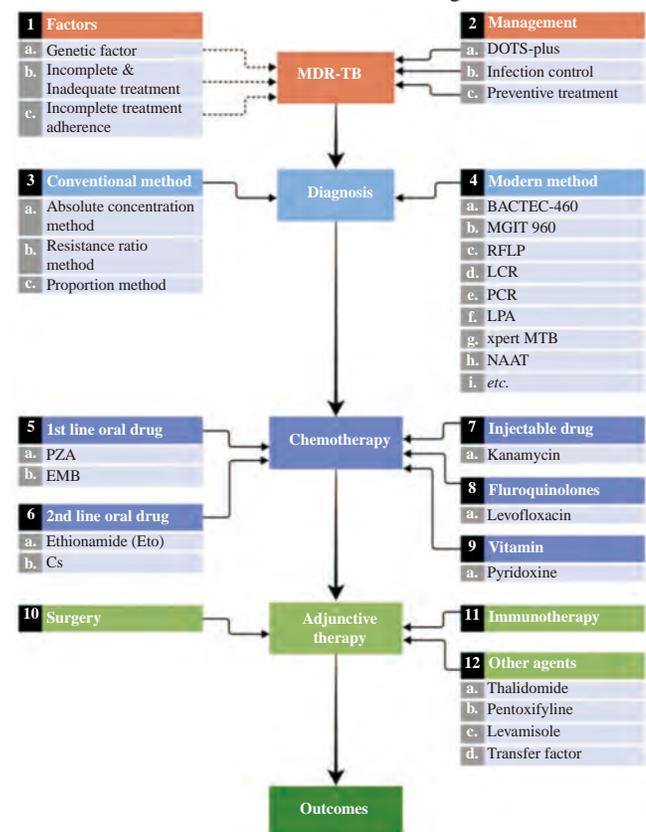


Figure 5. Schematic representation of the diagnostic and therapeutic management of MDR-TB.

MGIT: Mycobacteria growth indicator tube; RFLP: Restriction fragment length polymorphism; LCR: Ligase chain reaction; LPA: Line probe assay; NAAT: Nucleic acid amplification test.

A working group is established by WHO for MDR-TB on DOTS-plus, which develops policy guidelines towards the management

of MDR-TB. To enhance the availability and appropriate usage of the second line drugs for the treatment of MDR-TB, a Green Light Committee was also formed and established by the WHO[61,64]. Established DOTS-plus programs are proving to be beneficial for MDR-TB patients and also all patients with tuberculosis.

5.1. Diagnosis of MDR-TB

5.1.1. Conventional methods

Traditionally, for drug sensitivity testing by conventional method, Lowenstein Jensen media has been used. It is a lengthy procedure requiring 42-56 days for the sensitivity testing results to be known by the conventional method[65].

5.1.1.1. Absolute concentration method

Carefully controlled inoculums of MTB are inoculated in the control media (Lowenstein Jensen) and drug containing media for determining the MIC of the drug. The lowest concentration of the drug which will inhibit growth is indicated to be the MIC[65].

5.1.1.2. The resistance ratio method

Isolates' MIC is expressed in times of MIC's multiple for the standard susceptible strain. The intra-laboratory variation as well as the inter-laboratory variation is avoided in this method[65].

5.1.1.3. The proportion method

The share of drug resistant population in the entire bacterial pool is determined by the ratio of colony numbers being cultured in medium containing the drugs and without them[65].

5.1.2. Modern methods

Modern methods are used to hasten up the process of drug resistance profile differentiation.

5.1.2.1. BACTEC 460

It is a radiometric method. 7H12 medium is used in the BACTEC-460 (BD, USA). The medium involves radioactive carbon (¹⁴Cpalmitic acid) labeled palmitic acid. When the fatty acids are metabolized by the mycobacteria, the discharged radioactive CO₂ is measured to monitor the growth of the pathogens. The BACTEC technique is the modification of the proportion method and within 10 days sensitivity results will be available[66].

5.1.2.2. MGIT 960

The MGIT system is a fluorimetric method. For detecting the disease and performing susceptibility testing of MTB, this method is a useful, quick and non-radioactive one. The device in the system detects mycobacterial growth. The principal of the system depends on the fluorescent emission that is produced by an oxygen sensitive compound present at the bottom of the tube containing the medium[67,68].

5.1.2.3. RFLP

RFLP techniques are applied to categorize isolated strains of MTB and also to evaluate their characteristics. It has enabled the evolutionary relationship between strains to be determined and the

likely source of a TB infection to be identified. DNA extracted from the cultured bacilli, is used for RFLP techniques[69].

5.1.2.4. LCR

LCR is a fast technique used to detect TB, which uses the DNA ligase. The enzyme, DNA ligase functions to adjoin the two strands of DNA double helix. It is only possible when the ends of the DNA are complementary. The process acts by detecting even a single nucleotide mismatch in the luciferase gene, responsible for the production of light in fireflies. The method can identify most strains within 48 h[70,71].

5.1.2.5. PCR

The PCR is a fast technique. It is used for the detection of TB as well as for the understanding of the genetic mechanism of mycobacterial drug resistance. It was used for recognizing both old and new mutations. Rigorous sequencing reactions are required in each isolate's case. So, for the routine detection of drug resistance mutations, this very method cannot be accepted readily[72].

5.1.2.6. LPA

The LPA technique rapidly detects the resistance for RIF. The technique implies reverse hybridization. A *rpoB* gene segment is amplified and therefore denatured. Then the biotinylated PCR amplicons are hybridized by the probes which are bound to nitrocellulose membrane. The identification of MTB and detection of *rpoB* mutations are performed by interpretation of beading pattern on the membrane strip[73].

Besides all these main techniques, other new techniques which are being used to diagnose MDR-TB are rapid bacteriophage-based test, GenoType MTBDRplus assay, Xpert MTB/RIF and other NAATs.

Many TB control programs are not sure about which drug susceptibility tests (DSTs) to be performed and the appropriate time to do it. The cost-effectiveness of conventional and rapid DSTs was recently assessed for previously diagnosed smear-positive TB cases. Regarding this, the approach to execute a quick susceptibility test to identify any kind resistance to INH or RIF aimed at every patient prior to the introduction of therapy has been anticipated to be the best economical tactic[74]. These observations made the introduction of rapid DSTs to sustain at the moment of diagnosis to identify any kind of INH or RIF resistance in any country with sufficient loads of MDR- or XDR-TB.

5.2. Chemotherapy of drug resistant TB

Conventionally, the anti-TB drugs have been allocated into the first line drugs and the second line drugs. The INH, RIF, PZA, EMB and STR are categorized as the primary first line drugs. These drugs can also be grouped based on efficacy, experience of use and drug class. The different groups of drugs are shown in Table 2.

Management of MDR-TB with chemotherapy varies with the pattern of drug resistance. Generally, chemotherapy of MDR-TB demands treatment for 18–24 months using a regimen that contains 4–5 active drugs[75]. The regimen should include the use of any first line drugs against which resistance has not yet developed in addition to an injectable, a fluoroquinolone and other second line drugs as

Table 2

Four groups of drug regimen with orderly arranged drug names in each group.

Grouping	Drugs
First line oral anti-TB agent	INH, RIF, PZA, EMB
Injectable anti-TB agents	Capreomycin, kanamycin, amikacin, streptomycin, viomycin
Fluoroquinolones	Levofloxacin, moxifloxacin, ofloxacin, gatifloxacin, ciprofloxacin
Second line oral anti-TB agent	ETH, CS, <i>p</i> -aminosalicylic acid, prothionamide, terizadone, thiacetazone

needed to construct the 4 or 5 drug regimen. Once the isolated strain is established to be a multidrug resistant one, then based on the known sensitivities 5 remedies should therefore be picked to use in the following order (Table 3).

Table 3

Order of the drugs to be chosen based on sensitivities for MDR-TB treatment

Order	Drugs to be chosen
1	An amino glycoside (amikacin, kanamycin) or polypeptide antibiotic (capreomycin)
2	PZA
3	EMB
4	A fluoroquinolones: moxifloxacin is preferred (ciprofloxacin should no longer be used)[76]
5	Rifabutin
6	CS
7	ETH
8	<i>P</i> -amino salicylic acid
9	A macrolide (clarithromycin)
10	Linezolid
11	High-dose INH (if low-level resistance)
12	Interferon- γ [77]
13	Thioridazine
14	Meropenem and clavulanic acid[78,79]

Drugs have been arranged in the list from top to bottom according to efficacy and toxicity of them. This started with higher efficacy and less toxicity and ended with lower efficacy and higher toxicity and also the latter are hard to find.

Once four of the total drug regimen are already in hand, the addition of any one or both from vitamin D and arginine may prove to be advantageous for the treatment of MDR-TB. Arginine is beneficial as an adjuvant treatment, most likely facilitated by an increased production of nitric oxide, in HIV-negative patients with active TB whereas[80], a similarly important role is played by the vitamin-D regulated production of nitric oxide in the defense against the pathogenicity of the bacterium[81]; but you still need another drug to make five.

To strengthen and improve tuberculosis control activities, the Government of India launched the RNTCP in a phased manner. The MDR-TB treatment is considered as a 'standard care issue' by the RNTCP. The MDR-TB-treatment have recently been planned to abide by the guidelines of WHO for DOTS plus *i.e.*, to be done in the DOTS plus sites which have easy access to any of the RNTCP accredited drug susceptibility testing laboratories only to overcome the complexity of the treatment profile[82].

A standardized treatment regimen under the RNTCP program comprises of 6 drugs (kanamycin, levofloxacin, ETH, PZA, EMB, and CS) during 6-9 months of the intensive phase and 4 drugs (levofloxacin, ETH, EMB, and CS) during 18 months of the continuation phase for the treatment of MDR-TB cases. In this regimen, PAS is incorporated in the drug regimen as a substitute for any of the mycobactericidal drugs (levofloxacin, PZA, ETH or kanamycin) or any two other cidal drugs (EMB, CS)[83].

5.3. Adjunctive therapy in MDR-TB and XDR-TB

5.3.1. Surgery

In addition to drug based therapeutic measures, randomized controlled surgical programs are challenging to be organized towards the management of drug resistant TB. Surgical treatments in certain instances of MDR-TB are found to be beneficial though. Adjunctive surgery may be considered in the following conditions[84]: 1) Elevated risk failure or disease relapse with drug based therapy only because of wide-ranging bacillary resistance pattern; 2) Sufficiently localized infection, combined with ample fall back of lung function and the post-resection persistent ailment is submissive to clinical intervention; 3) Still, for the healing of the bronchial stump through reduction of the far-reaching bacillary burden, anti-TB agents are still not available in sufficient numbers.

The proper precautions must be taken before any operative measures, only after consultation with expert thoracic surgeons. Amongst them, rendering the sputum culture negative with the help of chemotherapy for at least three months is highly emphasized and the optimized nutritional status of the patient is desired as well[85,86]. Cardiopulmonary function, CT scans and other thoracic imaging studies are also included in the list of pre-operative measures to be looked after. In spite of having some risk factors, in case of operation, the life expectancy is much improved when compared to that with drug treatment only. But, to acquire the comparatively better health condition one has to simultaneously continue extensively the drug regimen after operative measures also. Otherwise, recurrence of the diseased condition might be a matter of time[87].

5.3.2. Immunotherapy

Alongside developing new mycobactericidal drugs, an effective investigation in search of potential immunotherapy in the form of complementary treatment is being done for a rapid recovery from the illness and avoiding relapse[88,89]. The protective immune mechanism against the pathogen includes cells, like macrophages and T-lymphocytes and regulatory proteins like cytokines that are elevated by these cells. Investigations regarding cytokine proteins have passed its way from preclinical to clinical stage, especially for IL 2 and 12 and interferons (IFN)- α and - γ . Previous investigations in the field have revealed considerable lowering of the bacillary load and progress in radiographic diagnosis, with the use of IFN- γ and an unusual mycobacterial vaccine *Mycobacterium vaccae*[90-96].

The significance of other capable immune-modulators, like vitamin-D seemingly demands more illustration regarding their efficacy and tolerance[97]. Various forms of vitamin D were also used successfully in scrofula and abdominal TB and with varying results in pulmonary TB[98-100].

5.3.3. Other agents

Several compounds have been at the center of interest of the research community as to act as effective adjunctive therapy for

MDR-TB patients. Thalidomide and pentoxifylline have been shown to combat the excessive effects from tumor necrosis factor- α [100-102]. These may be useful in limiting the wasting association with MDR-TB. Other agents which have occasionally been considered include, levamisole, transfer factor, inhibitors of transforming growth factor- β , IL-12 and imiquimod which is an IFN- α stimulating compound used as oral drug[103-107]. A contemporary study showed that usage of face masks by the MDR-TB patients could reduce the risk of nosocomial infection by at least 50%[108]. Literature survey has revealed that some new pyrazolo phenoxy acetic acid derivatives have shown moderate antitubercular efficacy and apart from this, a biological evaluation of 2-(4-arylthiazol-2-yl-amino)-n-aryl acetamides have also shown promising antitubercular activity[109,110]. Unlike all other previously used antibiotic agents, thioridazine activates the innate killing machinery of the non-killing human "hyper-acting" macrophage which would kill TB to a greater efficiency. Thus, the drug thioridazine, would cause efficient killing of TB with, likely, lack of generation resistance as TB's drug target biomolecules are not expected to be under a selective pressure[111,112].

5.4. Nutritional requirements in tuberculosis

By far the clinicians have successfully sorted out the nutritional consequences of the disease but a thorough knowledge regarding the interactions among patients' nutritional status and management of it and the treatment of the disease, is scarce as far as now. During the physiological response to the pathogenicity of the bacteria, the overall nutritional status involving the consumption and metabolism of the dietary intake is greatly changed. Ample nutrition is a significant aspect through all stages of disease. It has long been established that malnutrition is a threatening risk issue for becoming sick with TB[113]. Also it has to be understood that the disease itself is a threat to cause malnutrition and that even with the most suitable antibiotic treatment profiles the malnourished individuals with TB whose body mass index is lower than 18.5, remain at an increased death risk[114,115]. Therefore, it is well documented that a compromised immune system is linked to the patients' poor nutrition and a consistent observation and dietary assessment of the patient by a dietician are critical for the fruitful management of MDR-TB[116]. Providing free food probably does improve weight gain during treatment, and is thought to improve quality of life but further research is necessary[117].

5.4.1. Energy

Energy expenditure is increased during the body's attempt to fight the infection. The nutrient and energy needs for hyper catabolic and malnourished patients form the basis of existing suggestions for TB-infected persons (approx. 35-40 kcal/kg of body weight)[118].

5.4.2. Protein

The consumption of protein as food is key for preventing the wastage of muscles tissues by protein breakdown and high loss of protein (nitrogen). The consumption of approximately 75-100 g/day or 1.2-1.5 g/kg body weight is adequate[118].

5.4.3. Micronutrients

Any individual, infected with TB, requires increased levels of vitamin B6, C, D and E along with larger quantity of several minerals (iron, selenium, copper, zinc) and folic acid, all of which are vital in metabolic pathways involved in cellular functions including immune protection[119]. Therefore, it is even clearer now that the aforesaid micronutrients play major role in the host defence mechanisms during TB infection[114]. The physiological defence mechanisms could be compromised following insufficiency or lack of any of them. It has been observed that vitamins and minerals have key roles in potentiating our immune system to fight against the pathogen[120,121].

6. Clinical management of drug resistant-TB in patients co-infected with HIV

TB is one of the most common serious opportunistic infections in HIV positive patients. The lifetime risk of tuberculosis in immune competent persons is 5% to 10%, but in HIV positive individuals, there is a 5% to 15% annual risk of developing active TB disease[122]. TB can occur at any time during the course of HIV infection. In HIV infected patients, pulmonary TB is the most common form of infection and MDR-TB is also detected often in these cases[123]. Worldwide in 2010, 9 million people developed active TB, and 14.8% of the TB patients had HIV co-infection[1]. As a therapeutic measure, if possible, the treatment for HIV patients should be delayed until TB treatment is completed (CD4 count over 200). The data obtained in developed countries clearly demonstrate that the efficacious prevention of TB in adults with above 350 CD4⁺ cells/ μ L has its root to the substantial impact of antiretroviral therapy[124,125]. Immuno-compromised condition of HIV patients raises the likelihood of acquired drug resistance by reduced efficacy of anti-TB treatment regimens. The persons with AIDS and those with mal-absorption of anti-TB medications are generally observed to be diseased with high frequency. TB is the cause of death in one third of the population with AIDS worldwide. The management involving fast diagnosis, screening and prevention of drug resistant TB amongst the HIV co-infected population is a key issue towards eradication of the disease. Drug-resistant TB can be particularly lethal in patients with untreated HIV, with mortality rates as high as 98% in one series. Prompt identification of MDR and XDR-TB, initiation of effective TB treatment, and earliest initiation of antiretroviral therapy reduce the mortality attributable to drug-resistant TB in HIV co-infection[126,127]. The overlapping toxicity of HIV drugs and second line anti-TB drugs makes the situation even more complex to handle for the management of TB. In spite of the involvement of HIV infection with MDR-TB diseased condition being insignificant in India and other South-east Asian countries, the research community and clinicians might soon have to face the challenge of combating MDR-TB in association with HIV infection, if adequate measures are not taken with urgency[128]. HIV infection is a risk factor for the children who are exposed to or infected with TB. The HIV-positive children are far and away more prone to be diagnosed to acquire drug resistant TB than HIV-negative children. Gray *et al.* reported the ratio to be 6 to 1 child in 2009[129]. Data are limited regarding the use of preventative TB treatment in HIV

positive children^[130]. TB and HIV are independent threats and the combined effects are even worse^[131]. According to the latest data, when compared to the WHO estimates for India in 2010, the actual data regarding incidence of drug resistant cases seems to have exceeded with higher prevalence of the disease. And it is also plausible from the study that the prevalence of MDR-TB in HIV seropositive patients was significantly higher than seronegative individuals^[132].

7. New anti-TB drugs

In recent years of anti-TB drug development, research interests have been targeted towards combating genetically linked bacillary resistance and phenotypic perseverance. Several new agents with adequate potency in TB therapy are under careful observation^[133]. In connection with the report from the Working Group on New Drugs in 2008, it was estimated that more than sixty projects were in the due course, including clinical trials, lead compound discovery and translational basic research as well^[134]. Presently, gatifloxacin and moxifloxacin (fluoroquinolone class of drugs) are in the third phase of clinical trials for drug-susceptible TB-therapy. Apart from this, two nitroimidazoles, PA-824 and OPC-67683 and one diarylquinoline, TMC207 are in the second phase of their clinical trials. The three novel anti-TB agents are targeted towards the healing of both drug resistant and drug susceptible form of pathogen derived disease. Additionally, a pyrrole (LL-3858) and an ethylenediamine (SQ109) compound are in the preliminary stages of development^[133]. It is essentially important to accelerate the investigation to find more innovative ways and newer classes of agents whose efficacy sustain without discriminating phenotypic drug persistence and genetic drug resistance. Examples of some emerging compounds in various stages of clinical trials, include pleuromutilins, meropenem-clavulanate, nitrofuranyl amides, malate synthase inhibitors and several others^[133-136].

Discovery of new anti-TB drug is extremely difficult. Despite efforts of many decades by world/national health agencies as well as that of multinational pharmaceuticals such as Glaxo-Smith-Kline, Roche, and others towards development of new anti-TB drugs little was achieved. Recently, a drug, namely, bedaquiline has been developed by a Belgian company which apparently demonstrated (45 years after RIF's anti-TB activity was first effective against Indian patients) in a study led by Dr. Zarir Udhwadia of Hinduja Hospital. His publication published in *Clinical Infectious Diseases* issue of Jan 2012 produced intense enthusiasm in the TB-research community^[137]. The Government of India decided to introduce treatment plans including bedaquiline in the Mumbai metro areas, which is the world capital of TB, especially MDR-TB. Even a patient of Dr. Udhwadia reportedly got cured of her extremely dreaded XDR-TB infection which was acquired during her trip to Oman.

However, bedaquiline-related anti-TB regimen has not yet been approved by healthcare agencies of the Government of India due to the prohibitive cost of the drug and a lack of large scale trials that would likely establish the curative effect of that particular therapeutic agent. Also, MDR's frequency outside of the Mumbai metro area is not so alarming yet to necessitate the introduction of bedaquiline into the drug regimen. Also, considering the potential side effects of this

relatively new drug with a not-so-clearly elucidated mode of action, the Government decided not to fund a bedaquiline trial. A new anti-TB drug, delamanid (OPC-67683), a nitro-dihydro-imidazooxazole derivative, inhibits mycolic acid synthesis and this drug resulted in an increased negative culture-scoring of sputum at bimonthly interval among MDR-TB patients^[138,139]. Still, in conclusion, discovery of all these antituberculous agents could turn out to be a milestone in fighting TB worldwide, if all the yet-unknown factors hold good for these agents.

8. Future direction and conclusion

Tuberculosis has again emerged as a major health problem. Globally, it is the second most fatal contagious disease. It is essentially an obligation to understand the pathophysiology of the transmissible air-borne disease from the primary infection to the active form of it or latency. Acquired resistance accounts for the maximum drug resistance cases and this in turn increases the overall incidence of drug resistant tuberculosis. In India, MDR-TB occurrences are observed very rarely in most areas. More data on drug resistance have become available and estimates of the global MDR-TB burden have been improved. HIV infected individuals are in an added risk to develop TB whether it may or may not be the drug resistant strain. Combating the severeness of the MDR-TB and XDR-TB through a useful controlled programme on DOTS and its expanded form DOTS-plus is of paramount importance. Treatment of MDR-TB is a challenge which should be undertaken by experienced clinicians at centers equipped with reliable laboratory service for mycobacterial culture and *in vitro* sensitivity testing. Both conventional methods and inexpensive modern methods should be applied for early detection and management of MDR-TB. Established MDR-TB requires alternative and specific drug development to replace the current drugs which are even costlier and have worsened toxicity when used regularly. Further illustration is required in the drug resistant-TB management, regarding the roles of immunotherapy and surgical options. Discovery of new drugs would not only help in the prevention and therapy of the disease but will also shorten the total duration of treatment regimen by offering more effective therapy of latent tuberculosis infection. Further it has to be understood with better perspectives that provide food to both patients and the health care providers in the control programs would definitely benefit the programme itself by assuring the good nutritional status of both thus improving their performance. This is an effective way to increase the adherence towards the treatment of TB. Efforts have also been taken to develop alternative drugs from natural toxins (unpublished), which may be useful for the future management of MDR-TB.

Conflict of interest statement

We declare that we have no conflict of interest.

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