Extensively Drug Resistance Tuberculosis & Molecular Tools

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Abstract

Extensively drug-resistant tuberculosis (XDR-TB) is caused by bacteria that are resistant to some of the most effective anti-TB drugs. XDR-TB strains have arisen after the mismanagement of individuals with multidrug-resistant TB. Review article discuss about the Extensively drug-resistant tuberculosis w.r.t Indian Scenario.

Keywords: Non- tuberculosis mycobacterium, Multiple Drug Resistance, Extensively Drug Resistance, High-Resolution Melting.

Introduction

Mycobacterium tuberculosis is a gram-positive, aerobic, non motile, pleomorphic rods which causes tuberculosis, the leading cause of infectious disease mortality. An infectious disease caused by a bacteria and is confined to infection of the lungs, which can also spread to other parts of the body, such as the brain, kidneys, bones, tissues of the body, skin, intestine, meanings, joints and lymph nodes. The common symptoms includes coughing more than three weeks with blood-stained sputum, night sweats, fever, and weight loss (1). The way of transmission of this disease is air, when people who have the disease cough, sneeze (2). The bacterium Mycobacterium tuberculosis accounts for overall 90% of cases of tuberculosis.

M. tuberculosis, along with M. bovis, M. africanum, M. microti, M. Canetti, and M. intracellulare all are responsible for the disease known as Tuberculosis (TB) and are members of the tuberculosis species complex (Dormandy, T 1999). Each member of the TB
complex is pathogenic, but *M. tuberculosis* and *M. bovis* is pathogenic to humans and animals respectively. There are several species of mycobacterium that are collectively called Non-tuberculosis mycobacterium (NTM). NTM (M. avium, M. kansaii) causes neither Tuberculosis nor leprosy but a disease similar to that of tuberculosis, however Mycobacterium lepra is accountable for leprosy. Conventional, serological, biochemical and molecular methods are available for identification of mycobacteria. But most promising new approaches include molecular methods and its advancements for the detection of mycobacteria.

Most PCR assays used in the developing countries like India are based upon the PCR assays of developed countries. The PCR assays invented in the developed countries are based on the genetic make up of strains of Mycobacterial species available in those regions. There have been current reports that the Mycobacterial isolates from some geographical areas like the Indian subcontinent contain less copies of insertion sequence *IS6110* (many PCR assays are based on this gene sequence) as compared with the eight to 15 copies usually found in strains from most developed countries. As the number of copies of the target gene sequence is an important determinant of PCR sensitivity, it would be lower for strains having only a few copies of IS 6110. India accounts for a large proportion of tuberculosis cases, thus it had become necessary to evaluate PCR protocols based on the gene of *M. tuberculosis* in developing countries like ours. Amplification of a portion of DNA, which codes for a specific portion mannose binding protein 64 and is present only in the members of *M. tuberculosis* complex i.e. *M. Tuberculosis, M. bovis* and some strains of BCG of Indian subcontinent. A third of the world's population is thought to be infected with *M. tuberculosis*, and new infections occur at a rate of about one per second (3).

The proportion of people who become sick with tuberculosis every year is stable or falling worldwide but, due to population growth, the absolute number of new cases is still increasing (4). India and China alone accounts an estimated 35% of tuberculosis worldwide in 2008, an estimated 390000–510000 cases of MDR-TB emerged globally (best estimate, 440000 cases). Among all incident TB cases globally, 3.6% (95% confidence interval (CI): 3.0–4.4) are estimated to have MDR-TB. These estimates, which lie in the same range as the previous ones, are based on more data and a revised methodology) 150000 deaths In 2008 was due to MDR-TB. In 2007 there were an estimated 13.7 million chronic active cases, 9.3 million new cases, and 1.8 million deaths, mostly in developing countries (4). In addition, more people in the developed world are contracting tuberculosis because their immunity are compromised by immunosuppressive drugs, substance abuse, or AIDS.

Treatment for tuberculosis is difficult and it requires a long route of numerous antibiotics. Most infections in humans result in an undeveloped infection, and about one in ten undeveloped infections finally progresses to active disease, which, if left untreated, kills more than 50% of its victims. It is not the problem with developed countries as they have achieved inspiring results in the control of TB, it is the developing nations which account for about 95 per cent of TB cases, with Southeast Asia region, Africa and Western pacific being the worst affected regions. Several treatment protocols for active Tuberculosis are in wide use they generally have some basic principles like the regimen must include several drugs to which the organisms are susceptible, the patient must take the medication on a regular basis, therapy must continue for a sufficient time (*MMWR Morb Mortal Wkly 2003*), Unobtainable resources, poor socioeconomic conditions, individual immunocompetence, patient compliance, and complicated personal issues have also played roles in the evolution and progression of antibiotic resistance.

Different types of drugs are available for the treatment and control of tuberculosis. Isoniazid (INH) is one of the most common drugs used for TB. Inexpensive, effective and easy to take, it can prevent most cases of TB and, when used in conjunction with other drugs it can cure most TB. INH preventive treatment is recommended for individuals who have close contact with a person with infectious TB, positive tuberculin skin test reaction and an abnormal chest x-ray that suggests inactive TB, a tuberculin skin test that converted from negative to positive within the past two years ,a positive skin test reaction and a special medical condition (for example, AIDS or HIV infection or diabetes) or who are on corticosteroid therapy, Isoniazid and rifampin are the keystones of
treatment, but because of increasing resistance to them, pyrazinamide and either streptomycin sulfate or ethambutol HCL is added to regimens. If the patient is unable to take pyrazinamide, a nine-month regimen of isoniazid and rifampin is recommended (Centers for Disease Control and Prevention 2000).

TB requires much longer periods of treatment (around 6 to 12 months) to entirely eliminate mycobacteria from the body. Latent TB treatment usually uses a single antibiotic, while active TB disease is best treated with combinations of several antibiotics, to reduce the risk of the bacteria developing antibiotic resistance (O'Brien R, 1994). Treatment using Rifampicin and Pyrazinamide is not risk-free. The Centre for Disease Control (CDC) notified healthcare professionals of revised recommendations against the use of rifampin plus pyrazinamide for treatment of latent tuberculosis infection, due to high rates of hospitalization and death from liver injury associated with the combined use of these drugs (MMWR Morb Mortal 2003).

DOTS (Directly Observed Treatment, Short-course) are a strategy for the control of TB. It is based on research done in India over the past 40 years in order to eradicate all of the TB cases. The TB global emergency is further complicated by MDR- and XDR-TB strains that are resistant to our best antibiotics, very difficult to treat, and associated with greater morbidity and mortality than antibiotic susceptible TB. MDR tuberculosis is caused by Mycobacterium tuberculosis that is resistant at least to isoniazid and rifampicin, and XDR tuberculosis by mycobacteria resistant to rifampicin and isoniazid, any fluoroquinolone, and one of the three injectable drugs, capreomycin, kanamycin, and amikacin. First-line anti-TB antibiotics target actively replicating M. tuberculosis cells in the lung and significantly reduce transmission rates of M. tuberculosis to other persons within the first two months of treatment. The bactericidal antibiotics, isoniazid and rifampin, are active against dividing cells with rifampin also having activity against dormant bacteria, thus accounting for sterilizing properties during the short-course antibiotic regimen. The early bactericidal activity of drugs in patients with pulmonary tuberculosis (5).

Early bactericidal activity of isoniazid in pulmonary tuberculosis. Investigational models to explain the high sterilizing activity of rifampin in the chemotherapy of tuberculosis (6). Pyrazinamide exhibits utmost action against dormant organisms restricted within macrophages or the acidic environment of the pulmonary caseous lesion (7). Inclusion of ethambutol in the first-line drug regimen is recommended to avert rifampin resistance when isoniazid resistance is assumed (8). Second-line antibiotics are introduced into treatment regimens when resistance to primary antibiotics emerges. However, secondary antibiotics exhibit poorer effectiveness and/or higher toxicity (9). The fluoroquinolone, capreomycin and aminoglycoside antibiotics target DNA replication and protein synthesis and present the absolute efficacy of the second-line anti-Tuberculosis drugs (10). Programmes and principles in treatment of multidrug-resistant tuberculosis. The remaining antibiotics show bacteriostatic action and are significantly fewer potent, more poisonous, and more costly (11). Fortunately, in 2000, the WHO and its partners established the Green Light Committee Initiative which allows countries access to concessionally-priced, second-line anti-TB drugs for treating individuals with MDR-TB in accordance with WHO guidelines (12). Earlier to the accessibility of successful antibiotics, surgical intervention was a significant shape of treatment for pulmonary Tuberculosis (13).

An individual may develop the drug resistant form of TB via inadequate therapy that enables the selection of drug-resistance (acquired resistance) or infection with a drug-resistant TB strain (primary resistance) (14). While infection with an exogenous drug-resistant TB strain is related to infection control measures, the development of acquired M. tuberculosis resistance is multi-faceted and can be attributed to various social, political, economic, epidemiological, and pathophysiological factors (15). The drug resistance is a growing problem in the treatment of tuberculosis. Multidrug resistance tuberculosis have seen the widespread emergence of multidrug-resistant (MDR) tuberculosis, followed by extensively drug-resistant (XDR) tuberculosis and, most recently, strains that are resistant to all antituberculosis drugs. The tuberculosis control programme is severely threatened by drug resistance since it increases the possibility of a return to an era in which drugs are no longer effective. Numerous challenges have posed by XDR-TB in India, counting those of medical treatment and of TB control.
The high cost and low heal rate of treatment and nearly-100 per cent case fatality of XDR-TB are well-known.

During 1990’s, MDR TB emerged as a threat to TB control worldwide. XDR-TB or Extensively drug-resistant TB is a variety of MDR-TB that is also resistant to three or more of the six classes of second-line drugs firstly described in early 2006, following a joint assessment by the US Centre for Disease Control and Prevention (CDC), WHO and the Supranational Reference Laboratory Network (SRLN) as agreed upon by the WHO Global Task Force on XDR-TB. A frightening new abbreviation entered the medical glosary for the first time in March 2006. XDR stands for extensively drug resistant TB though some experts have it as extreme drug resistant TB. It was a report from the Center for Disease Control (CDC) and World Health Organization (WHO) in the March 24th issue of Mortality and Morbidity Weekly Report (MMWR) that first drew global attention to XDR TB. The original MMWR definition of XDR-TB was: resistance to INH and Rifampicin i.e. MDR-TB with further resistance to at least 3 of the 6 classes of 2nd line drugs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine and para amino salicylic acid. Drug resistant tuberculosis is transmitted in the same way as regular TB. Primary resistance occurs in persons who are infected with a resistant strain of TB.

A patient with fully susceptible TB develops secondary resistance (acquired resistance) during TB therapy because of inadequate treatment, not taking the prescribed regimen appropriately, or using low quality medication.[6] Statistics from Tuberculosis Research Centre (TRC), Chennai has documented that MDR TB gets fueled to a great degree by the formerly treated Tuberculosis cases (25%) in contrast to the new cases (3.4%). This observation leads belief to the outlook that the incidence of MDR TB is first and foremost a man made trouble. Other studies in India have also shown that the rates of acquired drug resistance are invariably higher than the rates of principal drug resistance (16). Further, emergence of XDR-TB will complicate the situation although as of now this is not being reported as a big problem for this country. However, one recent abstract presented in May at the American Thoracic Society’s 2007 conference claimed the prevalence of XDR-TB to be around 8% of all the MDR-TB cases. In India the report from the Hinduja National Hospital in Mumbai, said that of the 3,904 laboratory samples examined, 1,274 were positive for Mycobacterium tuberculosis. Around 32% of the positive samples were found to be MDR-TB, out of which 8% were XDR-TB. The death rate among the XDR-TB patients was 42%. Majority of patients with XDR-TB were of younger age group (averaging 30 years old), thus posing a major threat to our economically productive population (17).

The prevalence of drug-resistant tuberculosis in North India is known, but no serious efforts have been made to identify the drug resistance genotypes or their prevalence in the community. The present study was undertaken to characterize mutations prevalent in patient isolates of M. tuberculosis from North India with respect to a few of these drug target loci (18). TB prevention and control takes two parallel approaches. In the first, people with TB and their contacts are identified and then treated. Identification of infections often involves testing high-risk groups for TB. In the second approach, children are vaccinated to protect them from TB (19). TB requires much longer periods of treatment (around 6-12 months) to entirely get rid of mycobacteria from body. Resistance to anti-TB drugs occurs when these drugs are misused or mismanaged e.g. when patient do not complete their full course of treatment, wrong dose or length of time for taking the drugs, poor quality drugs taken etc. A strong laboratory network with the surveillance system in place is also urgently needed. TB drug sensitivity testing is one of the most neglected aspects of TB control (20) In India, there has been paucity of reports due to lack or limited access to testing drug susceptibility. Therefore, much of the drug resistance has been presumed clinically.

The available literature indicates that primary drug resistance is mainly to isoniazid. That too is also of varying order, but less than 20 per cent. Initial multi-drug resistance is probably very low MDR-TB is more common in previously treated patients. It constitutes 33-35 per cent of patients who have failed with rifampicin containing regimen. Acquired resistance to rifampicin (33-35 per cent) and isoniazid (50-55 per cent) is substantial. Strains resistant to rifampicin were usually resistant to isoniazid, where as converse was not necessarily true. Few strains resist almost all known anti-TB drugs. The gravity of the XDR -TB has
not escaped the keen attention of the WHO. The director of the stop TB treatment Mario Raviglione said: “either we intervene rapidly to stop the spread of this strain or you can foresee in the future that this strain will replace the other one. That would make it practically uncontrollable.” we in India need to take this prophetic words seriously.

Diagnosis and treatment contribute much to TB control programmes. Molecular methods are also being extensively used for understanding the mechanisms of drug resistance in tuberculosis. Specificity and speed are major advantages of molecular assay. In case of tuberculosis, mutation in the target sites, are considered most important mechanism of isolate becoming resistant. With the advancements in molecular techniques and their efficacy harnessing of the same is of utmost priority. New technique like HRM is the gold standard for the detection of mutations in the genes responsible for drug resistance tuberculosis. HRM is a rapid, accurate, simple, novel, homogeneous, post-PCR method, closed-tubed and low-cost method enabling genomic researchers to analyze genetic variations (SNPs, mutations, methylations) in PCR amplicons. HRM characterizes nucleic acid samples based on their disassociation (melting) behavior. Samples can be discriminated according to their sequence, length, GC content or strand complementarity. Even single base changes such as SNPs (single nucleotide polymorphisms) can be readily identified. The most important High Resolution Melting application is gene scanning - the search for the presence of unknown variations in PCR amplicons prior to or as an alternative to sequencing.

Mutations in PCR products are detectable by High Resolution Melting because they change the shape of DNA melting curves. A combination of new-generation DNA dyes, high-end instrumentation and sophisticated analysis software allows to detect these changes and to derive information about the underlying sequence constellation. It will be thus an ideal assay to be used in countries like India with a high prevalence of drug-resistant MTB and where cost-effectiveness is essential. As a mutation-scanning assay for detecting drug-resistant MTB, it can potentially lead to better treatment outcomes resulting from earlier treatment with the appropriate antibiotics (21). HRM technique represented an inexpensive, highly sensitive and high-throughput method to facilitate the screening of large numbers of clinical samples for epidemiological studies of drug-resistance of M. tuberculosis, especially in developing countries (22).

Mutation scanning techniques are used to detect sequence variants without the need for prior knowledge of the identity or precise location of the variant, in contrast with genotyping techniques, which determine the status of a specific variant. High-resolution melting is a recently developed method that shows great potential as a mutation scanning technique. Sensitivity and specificity for mutation detection are extremely high and the technique also has advantages of cost and throughput (23). Based on this information, more precise and rapid molecular testing can be developed and lead to more appropriate and timely treatment regimens.

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Conflict of Interest: None

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