International Journal of Research and Review
www.gkpublication.in E-ISSN: 2349-9788; P-ISSN: 2454-2237

Review Article

Multi Drug Resistant Tuberculosis: An Emerging Disease in Today's Scenario of Kashmir (J&K), India

Zafar Nowshad[1], Vandana Shrivastava[2], Abhishek Mathur[3], Ruchita[4]

[1]Dept. of Microbiology, Himalayan University, Arunachal Pradesh, India,
[2]Dept. of Microbiology, ITS Dental College, Ghaziabad, UP, India,
[3]National Centre of Fungal Taxonomy (NCFT) & EBEC, New Delhi, India,
[4]Hindu College of Pharmacy, Sonepat, Haryana, India.

Corresponding Author: Zafar Nowshad

ABSTRACT

Multidrug-resistant tuberculosis (MDR-TB) poses a formidable challenge to TB control due to its complex diagnostic and treatment challenges. The annual global MDR-TB burden is estimated at around 490,000 cases, or 5% of the global TB burden, however, less than 5% of existing MDR-TB patients are currently being diagnosed as a result of serious laboratory constraints. Alarming increase in MDR-TB, the emergence of extensively drug resistant TB (XDR-TB), potential institutional transmission, and rapid mortality of MDR-TB and XDR-TB patient with HIV co-infection, have highlighted the urgency for rapid screening, methods. Conventional methods for myco-bacteriological culture and drug susceptibility testing are slow and cumbersome, requiring sequential procedures for isolation of mycobacterium from clinical specimens, identification of Mycobacterium tuberculosis complex, and in vitro testing of strains susceptibility to anti-TB drugs. Rifampicin is a first line anti Tuberculosis drug active against bacilli in logarithmic and stationary phase, which interferes with RNA synthesis by binding to bacterial RNA polymerase. Tuberculosis bacilli achieve resistance to rifampicin by accumulation of mutations in a short-81bp region of the rpoB gene. Among many mutations identified in the rpo B gene, few were verified by molecular genetic methods as responsible for resistance to rifampicin. In this study, 8-different mutations were identified in an 81-bp section of a "hot spot" region of rpoB gene of rifampicin resistant strain of Mycobacterium tuberculosis. The clinical strains were evaluated in respect to drug resistance. It was found that the mutations in positions 526 (H/D) 516 (D/V) and 531(S/L) codons result in high level resistance to rifampicin. Mutations in position 516(D/Y) 515(M/I) 510 (Q/H) or a double mutation in codons 512(S/I) and 516 (D/G) relates to low level of resistance. The present study was performed in order to compare the isolation and drug sensitivity testing (DST) methods for Mycobacterium tuberculosis culture using solid media (Lowenstein-Jensen/LJ) and liquid media (BACTEC Mycobacterium growth indicator Tube-MGIT 960). This was a cross-sectional survey of adults who visited Intermediate Reference Laboratory, Srinagar (J&K), India with new diagnosis of pulmonary tuberculosis (TB) or failing the first- line TB treatment. Patients were requested to provide two sputum specimens for smear-microscopy and culture on solid and liquid media. Amongst 854 samples, 642 (75.17%) were positive, 211(25%) were found negative and 1(0.1%) were non-tuberculosis mycobacterium (NTM) when isolated through solid/LJ media while 735 (86.06%) were positive, 100 (12%) were negative and 19 (2.22%) were found NTM when isolated through liquid/BACTEC-GIT-960 media. Amongst the two media for isolation of Mycobacterium in random screening procedures, liquid media/BACTEC-MGIT-960 increases diagnosis of TB-positive samples and specifically those with MDR-TB. The choice of culture method should also depend on local availability, cost and test performance characteristics. It was found that, positive cultures of TB were found to be most resistant against streptomycin and most sensitive to ethambutol. The pattern of resistance against drugs in

Mycobacterium tuberculosis as per the study follows the order viz. streptomycin>isoniazid>rifampicin>ethambutol. The pattern of sensitivity follows the order viz. ethambutol>rifampicin>isoniazid>streptomycin.

Keywords: MDR TB, Kashmir valley, first line treatment, drug resistanc, solid/LJ media, liquid/BACTEC-GIT-960 media.

I. INTRODUCTION

Tuberculosis is an infectious disease caused by bacteria Mycobacterium tuberculosis. Tuberculosis is a killer disease, has probably been recognized since the Stone Age. Traces of Tuberculosis lesion have been found in the lungs of 3000 year Egyptian mummies. The Greek physician Hippocrates (450-370 BC)- "the father of medicine" wrote a description of the disease. The name tuberculosis appears to have been first used in 1839 by Johann Schonlein, In Classic Greek times it was known as phthisis. Till the present century, it was commonly called "consumption"- for the same reason. But it was in the 17th century that a Dutchman Franciscus silvious of Leyden first used the term "tubercle" to describe the knobby lesions found in the lungs of people who had died of the wasting disease. It was Dr. Robert Koch who identified Mycobacterium Tuberculosis by isolating the TB Bacillus which is rod shaped germ.

In India two patients dies every three minutes because of tuberculosis. Controlling TB is a tremendous challenge. The TB burden in India is still staggering. Every year, 1.8 million persons develop the disease, of which about 800,000 are infectious, and until recently, 370,000 died of it annualy-1,000 every day. The disease is a major barrier to social and economical development. An estimated 100 million work days are lost because of illness.

The bacteria can attack any part of your body, but they usually attack the lungs. TB disease was once the leading cause of death in world wide. In the 1940s, scientists discovered the first of several drugs now used to treat TB. As a result, TB slowly began to disappear in the world. But TB has come back. After 1984, the number of TB cases reported in the Asian countries began to increase. [1] TB is spread through the air from one person to another. But in other people specially people who have weak immune system the bacteria become active and cause TB diseases. The bacteria are put into the air when a person with TB disease of the lungs or throat coughs or sneezes. People nearby may breathe in these bacteria and become infected. When a man inhales TB bacteria, the bacteria can settle in the lungs and begin to grow. [2] From there, they move through the blood to other parts of the body, such as the kidney, spine, and brain. TB in the lungs or throat can be infectious. There is significant increase in the world wide incidence of TB. [2, 3] This means that the bacteria can be spread to other people. TB in other parts of the body, such as the kidney or spine, is usually not infectious. It has always been endemic and it is probably due to this complexity that the other mycobacteria commonly reported from other countries were not able to come up. Tuberculosis and HIV have been closely linked since the emergence of AIDS. HIV infection has contributed to a significant increase in the worldwide incidence of tuberculosis. By producing a progressive decline in cell-mediated immunity, HIV alters the pathogenesis of tuberculosis, greatly increasing the risk of developing disease in co-infected individuals and leading to more frequent extra-pulmonary involvement and atypical situation. [4] Although HIV-related tuberculosis is both treatable and preventable, incidence rates continue to climb in developing countries (like India, china, Africa etc.) where HIV infection and tuberculosis are endemic and resources are limited. Worldwide, tuberculosis is the most common opportunistic infection affecting HIV-seropositive individuals, and it is the most

common cause of death in patients with AIDs. There several other species of mycobacterium that are collectively called non-tuberculous mycobacterium (NTM). NTM (*M.avium, M.kansaii*) causes neither TB nor leprosy but a disease resembling that of TB. However *M. leprae* is responsible for leprosy. Tuberculosis is a major public health problem both in developed and developing countries. *Mycobacterium tuberculosis* (MTB) is responsible for most of the cases of tuberculosis. *M. tuberculosis*, along with *M. avium*, *M. africanum*, *M. microti*, *M. canetti*, all are responsible for the disease known as Tuberculosis (TB) and are members of tuberculosis species complex. There are several other species of mycobacterium that are collectively called Non-tuberculous mycobacteria (NTM). NTM causes neither TB nor leprosy but a disease resembling that of TB.

NTM also known as mycobacterium other than tuberculosis (MOTT) are the other mycobacteria which can cause pulmonary disease resembling tuberculosis, lymphadenitis, skin disease, disseminated disease, these are the most common clinical manifestation of NTM. Pulmonary NTM can also be found in individuals with AIDS and malignant disease. It is the most common cause of death in the patients with AIDS. [4] There are two main concerns about TB control in India and elsewhere: the rapid and sensitive diagnosis of the infection and identification of the pathogenic species. After AIDS epidemic, non-tubercular mycobacteria, especially *M. avium* complex have increasing been reported in these severely immune compromised patients. Not frequently, such patients will also have multiple infections with M. tuberculosis, *M. avium* and others Isolation of NTM from the respiratory tract does not, per se, indicate NTM has established diagnostic criteria to help distinguish between contamination and true NTM disease. Several reports of isolating *M. avium* concomitantly with *M. tuberculosis* as mixed infection in HIV positive patients have recently been published from all parts of the world. Infections with NTM should be considered in the differential diagnosis of any chronic infection, pyrexia of unknown origin and localized clinical disease (abscess, ulcers, nodules, infiltrates *etc*.) not responding to antibiotics. Attempt then should be made to repeatedly demonstrate and isolate the NTM from such lesions using most stringent criteria and precautions. Management of disease caused by non-tubercular mycobacteria or MOTT differ greatly from that of *M. tuberculosis*. Therefore, an early detection and identification of the infecting mycobacterial species are most desirable for early and specific therapy and better patient management, as PCR positive results without bacteriological confirmation of the specimen are doubted as false positive.

Therefore, we are going to standardize a multiplex PCR protocol for the differentiation and detection of *M. tuberculosis* (MTB) and MOTT in clinical specimens, which are based on molecular re approach. They have been increasingly recognized as pathogens in humans to cause the disease. As the culture with strict criteria is routinely done in most parts of India and there is a tendency to ignore such isolates as contaminants, it would be difficult to comment on the exact magnitude of the problem It is an anaerobic bacterium gram positive that divides every 16-18 hours and extremely slow rate compared with other bacteria. [5, 6] Though *M. tuberculosis* has been observed to be the main secondary infection in the reported cases of AIDS in India. The clinical presentation of the pulmonary disease due in the direction of NTM may be like tuberculosis' pulmonary and extra pulmonary infections. Non tuberculous mycobacteria were first identified in the late century, when a tuberculosis-like disease was found in chickens. The environmental opportunistic mycobacteria are normal In habitants of natural waters, drinking waters, and soils. They can be isolated from biofilms, aerosols, and dusts. They have even been recovered from potting soils and cigarettes. If present in water e.g., drinking water or

soil sample, they are not contaminants but rather are capable of persistence through growth. NTM causes neither TB nor leprosy but a disease resembling that a TB. [7] The distribution of NTM and the incidence of disease caused by them is perhaps not fully understood in most parts of the world. With the recent global resurgence of mycobacterial infection especially of tuberculosis, attributed to increased human immune deficiency virus infection, there is an increasing demand for rapid, sensitive, and specific diagnostic methods for the detection and identification of *Mycobacterium tuberculosis* and non tuberculous mycobacteria (NTM) in a clinical setting NTM infection can cause clinical problems, as its pathogenic potential and susceptibilities to anti tuberculosis treatments .Therefore, it has become important to be able to differentiate between the two during the early stage of the diagnostic procedure. Culture and microscope methods not being to differentiate between the MTB & NTM. [8] Nontuberculous mycobacteria (NTM), also known as environmental mycobacteria, atypical mycobacteria and mycobacteria other than tuberculosis (MOTT), are mycobacteria which do not cause tuberculosis or Hansen's disease (also known as leprosy): Nontuberculous mycobacteria (NTM) are all the other mycobacteria which can cause pulmonary disease resembling tuberculosis, lymphadenitis, skin disease, or disseminated disease. [5,6] In 1959, botanist Ernest Runyon put these human disease-associated bacteria into four groups (Runyon classification) which develop pigments in or after being exposed to light. Examples include *M. kansasii*, *M. simiae* and *M. marinum*.

Scotochromogens, become pigmented in darkness. Examples include *M. scrofulaceum* and *M. szulgai*. Non-chromogens, which includes a group of prevalent opportunistic pathogens called *M. avium* complex (MAC). Other examples are *M. ulcerans*, *M. xenopi*, *M. malmoense*, *M. terrae*, *M. haemophilum* and *M. genavense*. Rapid growers include four well recognized pathogenic rapidly growing non-chromogenic species: *M. chelonae*, *M. abscessus*, *M. fortuitum* and *M. peregrinum*. Other examples cause disease rarely, such as *M. smegmatis* and *M. flavescens*.The number of identified and cataloged NTM species has been increasing rapidly, from about 50 in 1997 to over 125 by January 2007. More that 90% of the TB causes death occurs in the world's 75% of the people are the most economically productive age groups. [7,8] The surge is mainly due to improved isolation and identification technique. However, even with these new techniques, the Runyon classification is still sometimes used to organize the mycobacteria into categories. NTM are widely distributed in the environment, particularly in wet soil, marshland, streams, rivers and estuaries. Different species of NTM prefer different types of environment.

Human disease is believed to be acquired from environmental exposures, and unlike tuberculosis and leprosy, there has been no evidence of animal-to-human or human-to-human transmission of NTM, hence the alternative label "environmental bacteria". .NTM diseases have been seen in most industrialized countries, where incidence rates vary from 1.0 to 1.8 cases per 100,000 persons. Recent studies, including one done in Ontario, Canada, suggest that incidence is much higher. Pulmonary NTM is estimated by some experts in the field to be at least ten times more common than TB in the U.S., with at least 150,000 cases per year. Most NTM disease cases involve the species MAC, *M. abscessus*, *M. fortuitum* and *M. kansasii*. *M. abscessus* is being seen with increasing frequency and is particularly difficult to treat. Mayo Clinic researchers found a three-fold increased incidence of cutaneous NTM infection between 1980 to 2009 in a population-based study of residents of Olmsted County, Minnesota. The most common species were M. marinum, accounting for 45% of cases and M.

chelonae and M. abscessus, together accounting for 32% of patients M. chelonae infection outbreaks, as a consequence of tattooing with infected ink, have been reported in the United Kingdom [9] and the United States. Rapidly growing NTMs are implicated in catheter infections, post-LASIK, skin and soft tissue (especially post-cosmetic surgery) and pulmonary infections.

The most common clinical manifestation of NTM disease is lung disease, but lymphatic, skin/soft tissue, and disseminated disease are also important. A typical Mycobacterial infection can cause infections such as abscesses, septic arthritis and osteomyelitis (bone infection), it can infect the lungs, lymph glands, skin or soft tissues.

Several species of Mycobacterium cause different infections. Mycobacterium avium intracellulare frequently affects AIDS patients. Mycobacterium marinum and M. ulcerans cause skin infections. M. marinum is responsible for swimming pool granuloma. M. avium-intracellulare and M. kansasii cause lung disease. M. scrofulaceum is a common cause of painless cervical lymphadenitis in children aged 1-5 years.

Pulmonary disease caused by NTM is most often seen in post-menopausal women. It is not uncommon for Cystic Fibrosis, Alpha-1 Antitrypsin Deficiency, Marfan's and Primary Ciliary Dyskenesia patients to have pulmonary NTM colonization a or infection. Pulmonary NTM can also be found in individuals with AIDS and malignant disease. It can be caused by many NTM species which depends on region, but most frequently MAC and *M. kansasii*. Lymphadenitis can be caused by various species that is different from one place to another, but again, MAC is the main cause worldwide. Most patient are aged less than 5 years, but the incidence is rare for children having BCG vaccine. The disease has a high curability. [9] Soft tissue disease due to NTM infection include post-traumatic abscesses (caused by rapid growers), swimming pool granuloma (caused by *M. marinum*) and Buruli ulcer (caused by *M. ulceran*sor *M. shinshuense*). Post-traumatic abscesses most commonly occur after injection. In 15–20% of active cases, the infection spreads outside the respiratory organs, causing other kinds of TB. These are collectively denoted as "extrapulmonary tuberculosis. Extrapulmonary TB occurs more commonly in immune-suppressed persons and young children. In those with HIV, this occurs in more than 50% of cases. Notable extrapulmonary infection sites include the pleura (in tuberculous pleurisy), the central nervous system (in tuberculous meningitis), the lymphatic system (in scrofula of the neck), the genitourinary system (in urogenital tuberculosis), and the bones and joints (in Pott's disease of the spine), among others. When it spreads to the bones, it is also known as "osseous tuberculosis" a form of osteomyelitis. Sometimes, bursting of a tubercular abscess through skin results in tuberculous ulcer. An ulcer origin nearby infected lymph nodes is painless, slowly enlarging and has an appearance of "wash leather. A potentially more serious, widespread form of TB is called "disseminated" TB, commonly known as military tuberculosis. [9] Disseminated mycobacterial disease was common in US and European AIDS patients in the 1980s and early 1990s, though the incidence has declined in developed nations since the introduction of highly active antiretroviral therapy. It can also occur in individuals after having renal transplantation. [9]

Diagnosis of opportunistic mycobacteria is made by repeated isolation and identification of the pathogen with compatible clinical and radiological features. Similar to *M. tuberculosis*, most non tuberculous mycobacteria can be detected microscopically and grow on Lowenstein-Jensen medium. Many reference centre now use a nucleic acid-based method such as sequence differences detection in the gene coding for 16S

Ribosomal RNA to identify the species. Pulmonary NTM disease diagnosis requires both identification of the mycobacterium in the patient's lung(s) as well as a high resolution CT scan of the lungs. The traditional diagnosis of mycobacterial infections based on culture and phenotypic identification is time consuming. Primary culture of slow growing mycobacteria on solid media usually takes 4-6 weeks and it is sometimes difficult to discriminate among closely related species Although molecular methods have not been able to replace culture for the detection of mycobacterial species in clinical specimens, their application combined with cultivation has accelerated the laboratory diagnosis of mycobacterial infections.

Recently, Polymerase Chain Reaction (PCR) or PCR linked methods have been used for rapid detection and differentiation of MTC and NTM. PCR is a sensitive method for detecting mycobacterial DNA or RNA directly in clinical specimens such as sputum, bronchial lavage, cerebrospinal fluid (CSF), pus, biopsy material, etc. Numerous PCR assays, which use conserved DNA or RNA sequences as targets for amplification, have been described for diagnosis of tuberculosis by detecting M.tuberculosis complex and mycobacteriosis. This technology has shortened test periods from several weeks to 1-2 days or even less. But none of these methods are universal due to region specific variations in the genome of mycobacteria. Considering that so far no single target sequence exploited has yielded 100% sensitivity and a total absence of false positive results when used alone. Multiplex PCR (mPCR) targeting of many different genes simultaneously has been used to detect and identify MTC and NTM in diagnostic laboratories However, some of these methods yield false negative results, as the target sequences (such as IS-6110) are not uniformly present in all clinical isolates. Although the development of DNA probes has greatly improved mycobacterial identification, particularly MTC, the commercial available Accu Probe DNA probe system .Besides being expensive, offers only a limited number of species-specific probes and the clinical isolates are mostly identified as MTC or NTM. The PCR- based reverse hybridization line probe assays (INNO-LiPA Mycobacteria and Genotype Mycobacteria) are expensive and their complex patterns make it difficult to implement them in a routine diagnostic laboratory. However, DNA sequencing of all culture isolates in a clinical diagnostic laboratory is not practical due to its prohibitive cost, particularly in resource-poor developing countries. The BCG strains differentiate not only genetically and phenol typically but also in their vaccine properties including tuberculin reactivity protective efficacy and prosperity of the adverse effect. [10] Thus, the present review was prepared to determine a simple, inexpensive, sensitive, reliable protocol for detecting and differentiation of MTC and NTM in clinical specimens. For this, mPCR based protocol must be innovated so that the disease must be diagnosed earlier with the other additional information within a single test run reaction. The protocol is based on molecular appraoch for the diagnosis purpose of the detection and discrimination between MTC and NTM or MOTT. Multiplex-PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes, i.e., their base pair length, should be different enough to form distinct bands when visualized by gel electrophoresis.

Biology of Mycobacterium

Tuberculosis complex organisms are obligate aerobes growing most successfully

in tissues with high oxygen content, such as the lungs. They are facultative intracellular pathogens usually infecting mononuclear phagocytes (e.g. macrophages), slow-growing with a generation time of 12 to 18 hours (c.f. 20-30 minutes for Escherichia coli), hydrophobic with a high lipid content in the cell wall. Because the cells are hydrophobic and tend to clump together, they are impermeable to the usual stains, e.g. Gram's stain, known as "acid-fast bacilli" because of their lipid-rich cell walls, which are relatively impermeable to various basic dyes unless the dyes are combined with phenol. Once stained, the cells resist decolorization with acidified organic solvents and are therefore called "acid-fast" (other bacteria, which also contain mycolic acids, such as Nocardia, can also exhibit this feature). Tuberculosis complex organisms are *Mycobacterium tuberculosis*, *Mycobacterium bovis*, (the cause of tuberculosis in cattle and humans, as well as other carnivores), *M. bovis* BCG (a strain used as a vaccine against tuberculosis in many parts of the world), and M. africanum (the cause of human tuberculosis in tropical Africa). In India, *Mycobacterium tuberculosis* causes tuberculosis in almost 100% of patients and hence, this manual aims at diagnosis of tuberculosis by culture of this organism.

Extent of Tuberculosis problem in India

The extent of TB Problem is generally described in terms of incidence, prevalence and mortality. Incidence is the number of new events (infection or disease) that occurs over a period of one year in a defined population. Prevalence is a total of new and existing events at a given point of time in a defined geographical population. India accounts for one fifth of the global TB burden i.e. 1.98 million out of 9.4 million new cases annually. In India, more than 40% population is infected with Mycobacterium tuberculosis. Approximately 75 new smear positive PTB cases occur per lakh population per year. it is also estimated that about 2,76,000 people die due to TB annually.

Tuberculosis prevalence in Kashmir valley

A tuberculosis prevalence survey was conducted in about 18,000 persons in Kashmir valley situated about 1650m above the sea level. All persons were tested with 3IU of PPD-S and 10 units of PPD-B. Such persons whose Montoux test was positive two sputum samples were collected and bacteriologically examined. The results of the survey showed that the prevalence of of non-specific sensitivity (59%) in the Kashmir valley is significant. The prevalence of the Tuberculosis infection was 38%.

According to data analysis done by State TB Demonstration cum Training centre, Srinagar, Kashmir, Jammu & Kashmir which are receiving monthly lab abstract from eight districts which have been designated as District Tuberculosis Centres by central TB Division Government of India includes Srinagar, budgam, pulwama, Kupwara, Anantnag, Baramulla, Kargil and Leh shows in first quarter 2013 the total number of suspects for TB were sent for sputum smear microscopy (Z-N Staining) is 14232 out of 617 were positive for Mycobacterium tuberculosis. . In the second quarter of 2013 13198 were sent for sputum smear microscopy out of 740 was detected as MTB Positive. In the third quarter 11385 patients were sent for sputum smear microscopy out of 742 was detected as Positive for MTB. Similarly in the fourth quarter 12027 patients were sent for sputum smear microscopies out of 584 were detected as positive for MTB. The patients were put on drug category first which includes streptomycin, isoniazid, rifampicin, ethambutol and PAS. The follow-ups of the patients who were on the treatment were conducted after three, five and six months. If the patient is still positive after three months this triggers to be suspect of MDR (Multiple drug resistant) the sample of this patient will be sent to the Intermediate reference laboratory for culture and drug sensitivity. [11-16]

Resistance in Mycobacterium tuberculosis

The mycobacterial cell is surrounded by a specialized highly hydrophobic cell wall that results in decreased permeability to many antimicrobial agents. Resistance of *M. tuberculosis* to anti-mycobacterial drugs is the consequence of naturally occurring, spontaneous mutations in genes that encode either the target of the drug or enzymes that are involved in drug activation. Resistance associated mutations have been described for all first line drugs (isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin).

Development of multi-drug resistance in Mycobacterium tuberculosis

No single genetic alteration has yet been found that results in MDR phenotype (defined as resistance at least to H and R) MDR develops as any sequential acquisition and selection of mutations at different loci, usually because of inappropriate patient treatment. Inappropriate treatment may lead to disease progression. Disease progression will increase the bacterial load and the risk of naturally occurring mutations. Because MDR strains are the results of cumulative mutation, growth of *M. tuberculosis* can successfully be controlled in the host by concomitant treatment with more than one drug. Thus treatment regimens that consist of three or four drugs are used routinely to treat patients with tuberculosis. H is a pro-drug that requires activation in H susceptible mycobacterial species. The activation of H results in a number of highly reactive compound that were capable of damaging the mycobacterial cell wall.

H resistant clinical isolates frequently lose their catalase – peroxidase activity Association of this enzyme with H activation was proven when the mycobacterial catalase – peroxidase gene was cloned and sequenced Mutations in this gene were found in 70- 80% of high H resistant clinical isolates. The most common mutation that was found was the Ser 315 Thr mutation. The Ser Thr mutation results in an enzyme without the ability to activate H but retains approximately 50% of its catalase – peroxidase activity. This altered catalase - peroxidase provides high level resistance to H while retaining a level of oxidative protection against host antibacterial radicals. Isolates that carry other mutations in Kat G exhibit varying levels of H resistance and catalase peroxidase activity. H blocks the synthesis of cell wall mycolic acids. Mutations in the promoter region of the gene (inh A) encoding this enzyme result in over expression of the protein. The over expressed enzyme may counter balance the effect of H and will result in a low level resistance to the drug. One of the main reasons for the treatment failure and fatal clinical outcome in tuberculosis patients is resistance to R. [17]

R exhibits a significant early bactericidal effect on metabolically active M. tuberculosis and excellent late sterilizing action on semi dormant organisms undergoing short bursts of metabolic activity. While mono-resistance to H is common, mono-resistance to R is rare. R resistance occurs most often in strains that are also resistant to H, thus surrogate marker for MDR Rifampicin resistance occurs most often in strains that are also resistant to H, thus it is a surrogate marker for MDR. R inhibits mycobacterial transcription by targeting DNA dependent RNA polymerase. Resistance to R is due to mutations in a well defined 81 base pair (27 codons) central region of the gene that encodes the beta subunit of RNA polymerase (rpo B). More than 96% of the rifampicin resistant strains contain a mutation in this 81 bp region of rpoB. The most common mutations (65-86%) alter either codon 526 or codon 531 and result in high level resistant to rifampicin. Alterations in other codons result in low level resistance. Rare mutations associated with rifampicin resistance have also been found in the amino terminal region of rpoB. (See Table 1, Figures 1-3).

Table 1: Molecular basis of Drug resistance in Mycobacterium tuberculosis:

Drug	Gene Locus	Gene function	Percent of Resistance
Isoniazid	*katG* *inhA* *ahpC*-Promoter	Catalase-Peroxidase Enoyl-ACP-Reduktase Alkyl-Hydroxid-Peroxidase	40 - 100 % appr. 25 % appr. 10 %
Rifampicin	*rpoB*	ß-Subunit of RNA-Polymerase	> 90 %
Pyrazinamide	*pncA*	Pyrazinamidase	appr. 95 %
Streptomycin	*rpsL* *rrs*	ribosomal Protein S12 16S rRNA	appr. 60 % appr. 20 %
Ethambutol	*embB*	Arabinosyl-Transferase	appr. 60 %
Chinolone	*gyrA*	DNA-Gyrase A	appr.80-90%

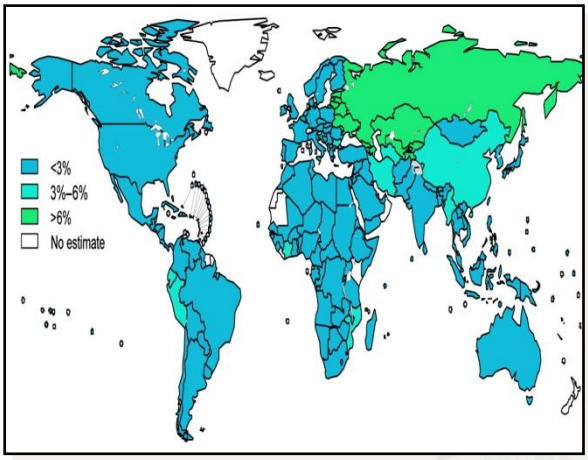

Figure 1: Distribution of MDR no prior treatment

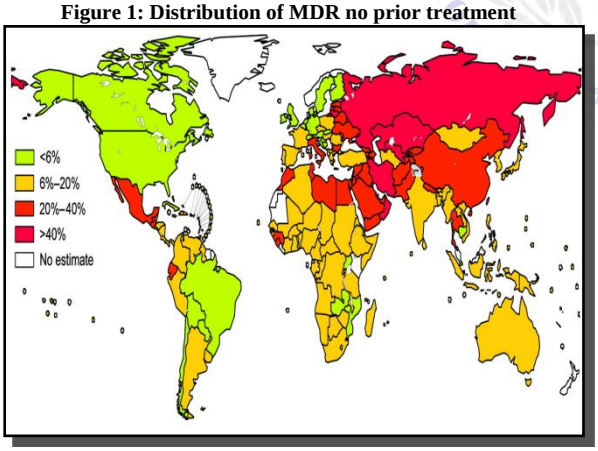

Figure 2: Distribution of MDR prior treatment

Genome

The Genome of Mycobacterium Tuberculosis has been sequenced with the help of going further understanding of how to defeat the infamously successful pathogens. The cell envelope of *M. tuberculosis*, a Gram-positive bacterium with a G + C-rich genome, M. tuberculosis sequence of 4,411,529 base pairs (bp) having a GC content of 65.6% genome contains an additional layer beyond the peptidoglycan that is exceptionally rich in unusual lipids, glycol-lipids and polysaccharides. [18] This represents the second-largest bacterial genome sequence currently available (Figure 4).

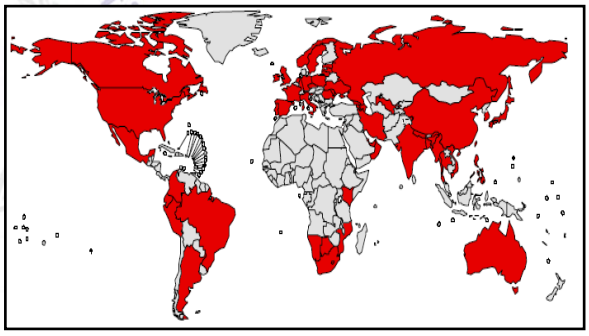

Figure 3: India MDR data (red marked)

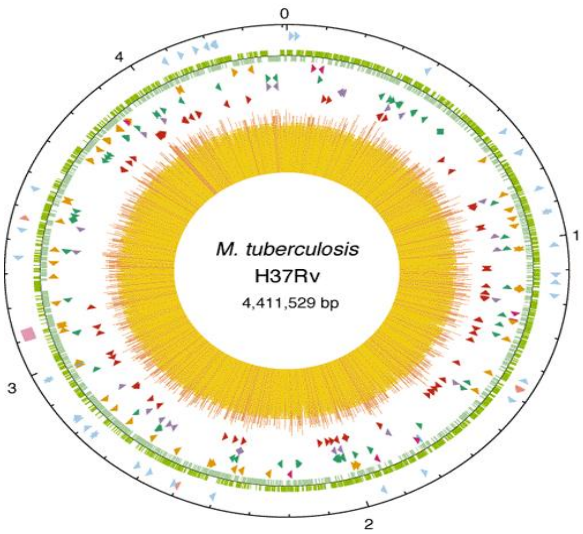

Figure 4: Circular map of the chromosome of *M. tuberculosis* H37Rv

Fifty genes coding for functional RNA molecules were found. The genes encoding tRNAs that recognize 43 of the 61 possible sense codons were distributed throughout. Three genes encoding tRNAs for methionine were found, one of these genes (*metV*) is situated in a region that may correspond to the terminus of replication. Sixteen copies of the promiscuous insertion sequence IS*6110* and six copies of the more stable element IS*1081* reside within the genome of H37Rv, 3924 ORFs have been identified accounting for ~ 91% of potential coding capacity . Host lipids have long been implicated as nutrient sources for *M. tuberculosis* during intracellular growth and chronic infection. Subsequently, sequencing of the *M. tuberculosis* genome revealed at least 250 genes potentially involved in lipid metabolism.By microscopic observation, Robert Koch first described the arrangement of bacilli in braided bunches and associated this phenomenon with virulent strains of *M. tuberculosis*. He also detailed the aspect of cultures in blood serum as compact scales which could be easily detached. In general, fresh virulent *M. tuberculosis* bacilli produce rough textured colonies on solid media expanded gummy veils on the surface of liquid media and serpentines on microscopic smears. In contrast, non-virulent mycobacteria and tubercle bacilli attenuated by prolonged cultures usually develop smooth colonies on solid media, form discrete mats in liquid media and distribute randomly in loose aggregates when got smeared.

Epidemiology of the Mycobacterium other than tuberculosis (MOTT) Infection

Although reports listing the significance of MOTT differ in various geographic parts of the world, there does seem to be a definite geographic distribution for some organisms. In the United States, MOTT lung disease is most commonly attributable to *M. avium* complex, with *M. kansasii* being second. In the United Kingdom, *M. kansasii* is the pathogen most commonly associated with MOTT lung disease in England and Wales, while *M. malmoense* is the most commonly encountered MOTT in Scotland. *M. xenopi* predominates in Southeast England. [19] In Japan, the most common cause of MOTT pulmonary disease the biological variation among MOTT is large when considering the ability to cause clinical disease in man and to affect various target organs or tissues.In contrast to *M. tuberculosis* and *M. leprae* that affect only mammals, MOTT form an integral part of the natural environment and may also prevail in certain man made environments, such as hot water tanks and tap water, thereby infecting and causing disease in vulnerable individuals. TB is one of the leading causes of mortality in India, killing two persons every three minutes, thus nearly 1,000 per day. [20] An estimated 2 billion people worldwide are infected with Mycobacterium tuberculosis (MTB), which remains a vast reservoir of potential tuberculosis cases. T.B is the major co infection in HIV patients. At present, about 5% of new tuberculosis cases in India occur in people infected with HIV infection. An extremely worrisome aspect of MTB is a recent rise in Multi Drug Resistance (MDR) MTB cases in several countries. New MDR MTB cases according to WHO estimates, constitute about 5% i.e. half million of nine million all types of TB. [19,20] In a study conducted on a cohort of patients from urban population near Mumbai recently, a very high (51%) incidence of MDR- MTB was reported. [15] MDR has now been recognized as a major public health problem that threatens success of DOTS, the WHO-recommended treatment approach for detection and cure of TB, as well as global tuberculosis control. The five countries that rank first to fifth in terms of total numbers of incident cases in 2008 are India (1.6–2.4 million), China (1.0–1.6 million), South Africa (0.38–0.57 million), Nigeria (0.37–0.55 million) and Indonesia (0.34–0.52 million). India and China alone account for an estimated 35% of TB cases

worldwide. The incidence of extra-pulmonary tuberculosis is higher in dialysis patients than in the general population. [21] Mycobacterial infection, mainly by *M. tuberculosis*, has an important impact on kidney transplant recipients, particularly during the first year after surgery. Environment and may also prevail in certain man made environments, such as hot water tanks and tap water, thereby infecting the most common cause of MOTT pulmonary disease the biological variation among MOTT is large when considering the ability to cause clinical disease in man and to affect various target organs or tissues.In contrast to *M. tuberculosis* and *M. leprae* that affect only mammals, MOTT form an integral part of the natural and causing disease in vulnerable individual.

Comparison of the MTC and NTM

Living and work environment has consistently been identified as an important risk factor for MOTT colonization and infection. Studies covering large geographic areas have generally found an increased risk of MOTT colonization in people living in warmer regions Living in urban versus rural settings has been associated with altered rates and patterns of MOTT colonization in several studies. The most commonly cited environmental risk factor for MOTT is the work environment, specifically mining, and other heavy industries such as smelting.

Also, residence in areas where these industries dominate may be a risk factor certain demographic features have been identified as risk factors for MOTT infection, including age, sex, or combination of the two.The main cause of TB is, Mycobacterium, a small aerobic non-motile bacillus or less commonly the closely related *Mycobacterium bovis.* [21,22] The high lipid content of this pathogen accounts for many of its unique clinical characteristics. It divides every 16 to 20 hours, an extremely slow rate compared with other bacteria, which usually divide in less than an hour. [22,23] Since MTB has a cell wall but lacks a phospho- lipid outer membrane, it is classified as a Gram-positive bacterium. However, if a Gram stain is performed, MTB either stains very weakly Gram-positive or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall. MTB can withstand weak disinfectants and survive in a dry state for weeks. In nature, the bacterium can grow only within the cells of a host organism, but M. tuberculosis can be cultured in the laboratory. The *M. tuberculosis* complex includes five other TB-causing mycobacteria: *M. bovis*, *M. africanum*, *M. canetti*, and *M. microti*.

M. africanum is not widespread, but in parts of Africa it is a significant cause of tuberculosis. M. bovis was once a common cause of tuberculosis, but the introduction of pasteurized milk has largely eliminated this as a public health problem in developed countries.

Pathogenesis

The distribution of MOTT and the incidence of MOTT diseases is still a paradox in most parts of the world however, consistent reports show that MOTT are ubiquitous in the environment and can colonize or infect people and animals, therefore, the environment is regarded to the biggest reservoir and source of MOTTs for animal and human hosts. In contrast to *M. tuberculosis* and *M. leprae*, species that affect only mammals, MOTT form an essential part of the natural environment and may also conquer in certain man-made Environments, thereby revealing susceptible individuals leading to colonization and infection. MOTT act like saprophytes, commensals, and symbionts and are common inhabitants of a wide variety of environmental reservoirs throughout the world, including natural and municipal water, soil, aerosols, protozoans, domestic- and wild animals, milk- and food products. MOTT may be abundant in certain natural surroundings or niches, where climatological factors are advantageous for their growth.

Infection by Mtb occurs via inhalation of 1-5 µm droplets containing one or several bacteria. These small

droplets deposit into alveolar airspace, while larger particles are efficiently cleared by the pulmonary mucociliary system. Infecting bacteria are phagocytosed by resident alveolar macrophages and can begin to replicate within the membrane-bound phagocytic vesicles. [23] Eventually the bacterial burden overwhelms the macrophages leading to the rupture of the cells and the release of numerous bacilli. These bacteria are then taken up by other alveolar macrophages and by monocyte-derived macrophages (MDMs) emigrating from the blood stream (Figure 5).

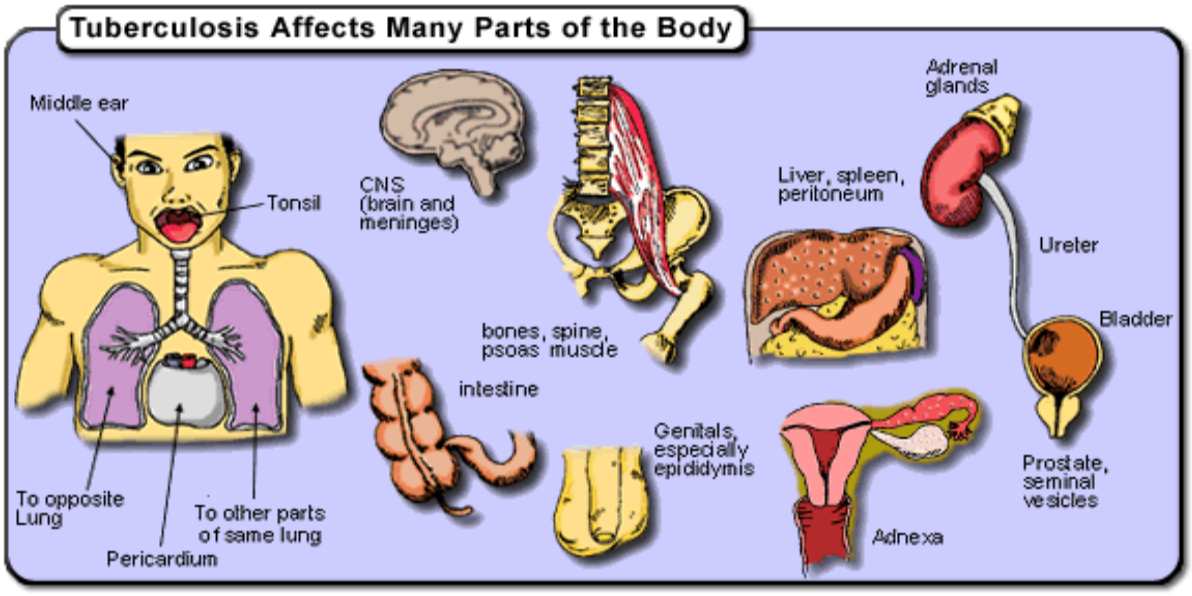

Figure 5: Tuberculosis affects many part of the body

Once in the lungs the bacteria are phagocytosed by macrophages and a hypersensitivity response forms small, hard nodules called tubercles, which are characteristic of tuberculosis and give the disease its name. The disease process usually stops at this stage, but the bacteria often remain alive within macrophage phagosomes. Resistance to oxidative killing, inhibition of phagosome-lysosome fusion, and inhibition of diffusion of lysosomal enzymes are some of the mechanisms that may explain the survival of MTB inside macrophages. By three weeks post-infection, a specific T-cell response emerges. Release of the lymphokines interferon and tumor necrosis factor-activates macrophages and there by checks bacterial replication. In nature bacterium can grow in only host organism but MTB can culture in the laboratory. [24,25] This spreading is often called miliary tuberculosis due to the many tubercles the size of millet seeds that are formed in the infected tissue. It also may be called reactivation tuberculosis because the bacteria have been reactivated in the initial site of infection. Persons infected with *M. tuberculosis* develop a cell-mediated immunity due to the bacteria being phagocytosed by macrophages. In Japan, environment and may also prevail in certain man made environments, such as hot water tanks and tap water, thereby infecting the most common cause of MOTT pulmonary disease the biological variation among MOTT is large when considering the ability to cause clinical disease in man and to affect various target organs or tissues. In contrast to *M. tuberculosis* and *M. leprae* that affect only mammals, MOTT form an integral part of the natural and causing disease in vulnerable individuals.

Diagnosis of atypical mycobacteria

Atypical mycobacteria are diagnosed on culture of tissue. Specific conditions are required, so the laboratory must be informed of the clinician's suspicion of this diagnosis.

The infections have specific pathological features on skin biopsy.

Signs and Tests:

- ✓ Blood culture.
- ✓ Sputum culture.
- ✓ Lymph node culture or biopsy.
- ✓ Bone marrow culture.
- ✓ Stool culture.
- ✓ Chest X-ray

Biochemical tests

Biochemical analysis for the differentiation within the MTC include nitrate reduction on modified Dubos broth, niacin accumulation test, growth in the presence of thiophen-2-carboxylic hydrazide (TCH), catalase activity at room temperature and growth characteristics on Lebek and on bromocresol purple medium. Previous methods relied mostly on biochemical tests to classify the species. [26] However, various laboratories use different methods and may also use different criteria to interpret results of the same test. For example, resistant to TCH is a feature of *M. africanum* however, the critical concentrations used for this test range from 1 to 5µg/ml. To determine oxygen preference, some laboratories use Lebek medium while others use Middlebrook 7H9 broth. This lack of standardization always creates ambiguity in interpretation of the results. Typically, in laboratories that use biochemical tests, only classical *M. tuberculosis* is thought to be resistant to TCH, likewise monodrug resistance to pyrazinamide (PZA) has been listed only for *M. bovis* and M. bovis BCG, and a preference for micro-aerophilic conditions differentiates *M. africanum* and *M. bovis* from other members of the complex. Interestingly, *M. bovis* was thought to be best identified by screening those isolates of the MTC that has any PZA resistance. It has recently been shown that the sensitivity for detection of *M. bovis* is lowered to 82% when only PZA mono resistant isolates are screened (Table 2, Figure 6).

Table 2: Clinical features of mycobacterium other than tuberculosis

Mycobacteria	Clinical features
Mycobacterium avium-intracellulare	Also known as MAC (Mycobacterium avium complex) Most common non-tuberculous mycobacterial infection associated with AIDS Symptoms include fever, swollen lymph nodes, diarrhoea, fatigue, weight loss and shortness of breath May develop into pulmonary MAC Skin lesions are uncommon
Mycobacterium kansasii	May cause a chronic infection of the lungs similar to pulmonary TB Second most common non-tuberculous mycobacterial infection associated with AIDS Symptoms include fever, swollen lymph nodes and lung crackles and wheezing Skin lesions may occur either alone or as part of a more widespread disease
Mycobacterium marinum	Also known as fish tank granulomas Uncommon infection that occurs most often in people with recreational or occupational exposure to contaminated freshwater or saltwater Usually a single lump or pustule that breaks down to form a crusty sore or abscess. Other lumps may occur around the initial lesion, particularly along the lines of lymphatic drainage (sporotrichoid forms) Most often affects elbows, knees, feet, knuckles or fingers Multiple lesions and widespread disease may occur in immune compromised patients Rarely causes red, swollen and tender joints
Mycobacterium ulcerans	Also known as Buruli ulcer Infection most common in Central and West Africa around areas of lush vegetation and swamps but may also occur in Australia Solitary, painless and sometimes itchy nodule of 1-2 cm develops about 7-14 days after infection through broken skin Over one to two months the nodule may break down to form a shallow ulcer that spreads rapidly and may involve up to 15% of the patient's skin surface Severe infections may destroy blood vessels, nerves, and invade bone
Mycobacterium chelonae	Worldwide distribution: found in tap water and other water sources May cause lung disease, joint infection, eye disease and other organ infections May result in non-healing wound, subcutaneous nodule or abscess Immunosuppression may cause disseminated lesions throughout the body

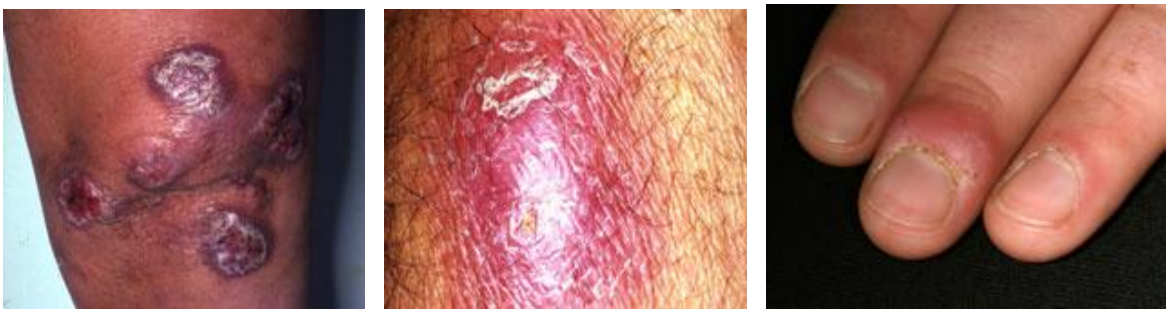

Figure 6: *Mycobacterium marinum* infection

Laboratory Diagnosis

Bacteria TB culture system for identification of MOTT: The following specimens may be checked:

- Blood
- Sputum
- Lymph node biopsies and aspirates
- Bone marrow
- Body fluids
- Stool

(A) Principle of the Test

NAP (p-nitro-a-acetyl amino-B-hydroxy-propiophenone) an intermediate compound in the synthesis of Chloramphenicol inhibits Mycobacteria belonging to the Tuberculosis complex, (*M.tuberculosis, M. bovis, M. africanum* and *M. microti*) almost completely, while other Mycobacteria show either slight or no inhibition. When Mycobacteria growth is inhibited in the presence of NAP, the evolution of CO2 is also inhibited, as indicated either by no increase or a decrease in the Growth Index. This effect on the GI is used as a tool for identification. The results are available within 2-6 days after putting up the test.

(B) Molecular method for identification of MOTT

Recently developed molecular methods, such as DNA probe test and PCR can also be used to differentiate between MTB complex and MOTT (Figure 7).

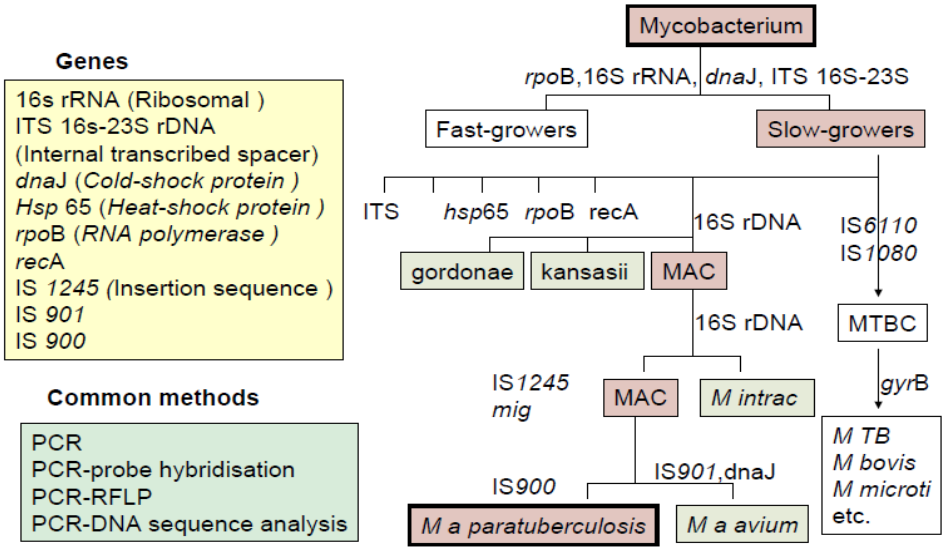

Figure 7: Differentiation of Molecular Mycobacterial Species

Molecular markers for the differentiation of MTB complex and MOTT complex

Recently, PCR or PCR linked methods have been used for rapid detection and differentiation of MTC and NTM. Multiplex PCR targeting of many different genes simultaneously has been used to detect and identify MTC and NTM in routine diagnostic laboratories. The DNA sequencing of mycobacterial gene targets

such as 16s rRNA, rpoB, hsp65, secA and 16S-23S internal transcribed spacer (ITS) region genes are used for species-specific identification of nearly all mycobacterium species. The results of mPCR for some randomly selected MTC isolates and all NTM strains were confirmed by PCR amplification of the 16S-23S ITS region followed by direct DNA sequencing of the amplified DNA. The ITS region was amplified by using pan-mycobacterial primers MYITSF and MYITSR. The amplicons of 350 bp to 500 bp obtained from various mycobacterial species (due to variable length of the ITS region) were sequenced using the same amplification primers. Multiplex PCR targeting the oxy R-ahpC intergenic region and rpoB gene were used for direct detection and differentiation of clinical isolates of MTC or NTM. The oxyR-ahpC genes are divergently transcribed in mycobacteria. M.tuberculosis and other members of MTC naturally contain defective oxyR due to mutations in the 5' end of the gene while NTM contain functional gene copy. [27] MTC specific primer sequences derived from the rpoB gene differ from the DNA sequences of the corresponding region from several NTM species by a single nucleotide at the 3' end. When PCR amplification was performed with IGRF and IGRR primers together with genomic DNA from various mycobacteriums or other bacterial species, an amplicon of 473bp was specifically obtained from MTC members only. Further, an amplicon of 235bp was obtained only from MTC members and not from NTM, when PCR amplification was carried out with primers MTCF and MTCR, while an amplicon of 136bp was obtained only from NTM but not from MTC members, when primers. NTMF and NTMR were used during PCR amplification. PCR was performed by using all six primers (IGRF, IGRR, MTCF, MTCR, NTMF, NTMR simultaneously, MTC members yielded two amplicons of 473bp and 235bp while all NTM yielded a single amplicons of 136bp. No amplicon was obtained from other bacterial or fungal species.

Treatment of atypical mycobacterium

Treatment of atypical mycobacterial infections depends upon the infecting organism and the severity of the infection. In most cases a course of antibiotics is necessary. These include rifampicin, ethambutol, isoniazid, minocycline, ciprofloxacin, clarithromycin, azithromycin and cotrimoxazole. Usually treatment consists of a combination of drugs.

Some points to consider when treating atypical mycobacterial infections:

- *Mycobacterium marinum* species are often resistant to isoniazid. Treatment with other antibiotics should be for at least two months.
- *Mycobacterium kansasii* should be treated for at least 18 months
- *Mycobacterium chelonae* is best treated by clarithromycin in combination with another agent, sometimes surgical excision is the best approach.

AIDS patients on HIV protease inhibitor drugs cannot be treated with rifampicin because rifampicin significantly increases the breakdown of these drugs. Rifabutin is a suitable alternative. Antibiotics are usually ineffective in treating large skin lesions caused by *Mycobacterium ulcerans*. Rifampicin may promote healing of pre-ulcerative lesions. Most lesions eventually spontaneously heal after 6-9 months but may leave behind extensive scarring and disfigurement. Surgical removal of infected lymph nodes and skin lesions is sometimes necessary. In severe cases, skin grafts may be necessary to repair the surgical wound.

The Primary Infection

Usually, pathogens enter the lung in droplets, where they are phagocytosed by alveolar macrophages. MTB are able to reproduce in these macrophages due to their ability to inhibit formation of the phagolysosome. Within 10–14 days a reactive inflammatory focus develops, the

so-called primary focus from which the TB bacteria move into the regional hilar lymph nodes, where they reproduce and stimulate a cellular immune response, which in turn results in clonal expansion of specific T lymphocytes and attendant lymph node swelling. When the host encounters with M. tuberculosis for the first time, the following events occur:

- Local inflammatory response - Encounter with neutrophils and resident macrophages.
- Killing of the phagocytosed tubercle bacilli occurs only when macrophages are activated. Simultaneously, the development of cell-mediated immunity, and delayed type hypersensitivity to tuberculin (antigenic protein(s) produced by M. tuberculosis.
- If the host defense system fails to contain the spread of the bacilli at this point, It is estimated that approximately 10% of individuals with normal immunity well develop active TB within their lifetime, 5% within the first 2 years of infection.
- Tubercle bacilli spread in the host by direct extension, through the lymphatic channels and bloodstream, and via the bronchi and gastrointestinal tract. These events result in tuberculous meningitis, miliary tuberculosis or both.
- If a caseating lesion discharges its contents into a bronchus, they are aspirated and distributed to other parts of the lungs and passed into the stomach and intestines.

Progressive primary infection

After the development of hypersensitivity, the infection becomes quiescent and asymptomatic in the majority of patients (about 90%). In some, however, especially the very young and adults who are immune-compromised or who have other predisposing illnesses, the primary infection may evolve into clinical disease. The progression may be local at the site of the primary lesion, or it may be at one or more distant sites where bacilli have arrived during the early haematogenous spread.

Secondary Infection

The characteristics of secondary tuberculosis include extensive tissue damages due to immunologic reactions of the host to tubercle bacilli and their products. In this phase of the disease, lesions are usually localized in the apices of the lungs (remember that tubercle bacilli require oxygen for growth). In about 5% of patients, apical pulmonary tuberculosis manifests itself within 2 years of the primary infection. In others, however, clinical disease may evolve many decades later whenever resistance is lowered. Quiescent foci that harbor viable organisms thus remain a potential hazard throughout a person's lifetime. Because of the acquired cellular immunity, bacilli are more promptly phagocytized and destroyed by the activated macrophage. As a result, in secondary tuberculosis, lesions remain localized and dissemination of organisms via the lymphatic vessels is usually prevented. Hypersensitivity promotes a more rapid caseation and fibrotic walling-off of the focus. Histologically, the reaction is characteristic of tubercle formation, manifested by a local accumulation of lymphocytes and macrophages. These differences between primary infection and post-primary or reactivation are attributed to (1) resistance and (2) hypersensitivity induced by the first infection of the host with tubercle bacilli The body's immune defenses have a hard time containing necrotic tissue lesions in which large numbers of TB cells occur (e.g., up to 10^9 bacteria and more per cavern), the resulting lymphogenous or haematogenous dissemination may result in infection foci in other organs. Virtually all types of organs and tissues are at risk for this kind of secondary TB infection. Such infection courses are subsumed under the term extra pulmonary tuberculosis.

Immune Response of the Host to M. tuberculosis

The alveolar macrophages, after entry of *M. tuberculosis*, produce inflammatory cytokines and chemokines that serve as a signal for infection. During this time, the bacilli resist the bactericidal mechanisms of the macrophage (phagolysosome) by preventing phagosome-lysosome fusion, multiply in the phagosome, and cause macrophage necrosis. The accumulation of macrophages, T cells, and other host cells (dendritic cells, fibroblasts, endothelial cells, and stromal cells) leads to the formation of granuloma at the site of infection. The granuloma formation walls off tubercle bacilli from the rest of the lung tissue, limits bacterial spread, and provide microenvironment for interactions among macrophages and other cells of the immune system and the cytokines produced by these cells. The $CD4^+$ T cells producing interferon-γ (IFN-γ) recognize infected macrophages presenting antigens from *M. tuberculosis* and kill them. The $CD4^+$ T cells carry out several functions that are important to control infection in the granuloma. These include apoptosis of infected macrophages through Fas/Fas ligand interaction, production of other cytokines (such as IL-2 and TNF-α), induction of other immune cells (macrophages or dendritic cells) to produce other immuno-regulatory cytokines such as IL-10, IL-12, and IL-15, and activation of macrophages through direct contact via CD40 ligand $CD4^+$ T cells can control the intracellular growth of *M. tuberculosis* by a nitric oxide-dependent mechanism that is independent of IFN-γ production The $CD8^+$ T-cells, in addition to producing IFN-γ and other cytokines, may also be crypto-toxic for *M. tuberculosis*-infected macrophages and thus play an important role in providing immunity to TB. The $CD8^+$ T-cells can directly kill *M. tuberculosis* via granulysin, and facilitate the control of both the acute as well as chronic infection. The IFN-γ is the key cytokine for a protective immune response against *M. tuberculosis*. The IFN-γ, produced mainly by $CD4^+$, $CD8^+$ T cells, and the NK cells, synergizes with TNF-α and activates macrophages to kill intracellular bacilli. The IFN-γ also augments antigen presentation, leading to recruitment of $CD4^+$ T-cells and/or cytotoxic $CD8^+$ T-cells, which participate in mycobacterial killing and also prevents exhaustion of memory T cells. TNF-α also initiates cell migration and formation of microbicidal granulomas while disruption of TNF-α response leads to overgrowth of the mycobacterial pathogens. [27] Both T cell- and macrophage-derived TNF-α are required for sufficient and long-term protection against *M. tuberculosis* infection.

Diagnosis

Of the 130 known species of mycobacteria, nearly one third have been observed to cause disease in humans. The most common sites where mycobacterial disease occurs are the lungs, the lymph nodes and skin. However, as *M. tuberculosis* is mostly known to cause the well-established pulmonary manifestation but is capable of infecting virtually all tissue types, the MOTT species follow the same behavior. [28] Pulmonary disease, lymphadenitis, and disseminated infection are the commonest and most important clinical problems but infection and disease do occur at other sites, such as the soft tissues, bone, joints, and genitourinary trac.

Microscopy

Laboratory diagnosis of TB has traditionally been based on smear microscopy. The quickest, easiest and cheapest method available is acid-fast staining; its low sensitivity has limited its usefulness, especially in geographical areas of lower incidence, in extra-pulmonary forms (paucibacillary) of TB, and in HIV-infected patients. It should also be noted that a significant percentage (17%) of transmission occurs from smear negative pulmonary tuberculosis patients. A further point is that despite having good overall specificity the smear has a low positive predictive value (50%-80%) in areas of higher incidence of non tuberculosis

mycobacteria (NTM) clinical isolates. To detect one to two organisms in 300 oil immersion fields, the concentration of organisms must be 5,000-10,000 per ml. Thus under operational conditions positive smears are found in 50-70 per cent specimen. Early and timely diagnosis of tuberculosis relies heavily on microscope examination of clinical samples for acid fast bacilli using the Z-N stain. Microscopy can detect 60-70 per cent of culture positive samples with a lower limit of detection of 5 x 10^3 organisms/ml, typical acid fast bacilli appear as slightly curved, beaded long or short rods.

Fluorochrome Stains

The waxy mycolic acids in the cell walls of mycobacteria have an affinity for the fluorochromes, auramines and rhodamine. These dyes non-specifically bind to nearly all mycobacteria. The mycobacterial cells appear bright yellow or orange against a greenish background. This method can be used to enhance detection of mycobacteria directly in patient's specimen. The advantage of this method is that lower magnification can be used, so wider area is covered enabling rapid screening

Culture Techniques

Culture techniques are still regarded as the reference method due to its sensitivity and the fact that further studies can be conducted with the isolated mycobacteria (identification, sensitivity and epidemiological typing)However, the slow growth of the tubercle bacillus is a major obstacle to rapid disease diagnosis. But, several weeks are still required to obtain the final laboratory confirmation, and even longer in the case of conventional phenotypic identification procedures.

Solid medium-based methods

Culture of mycobacteria is a much more sensitive test than smear examination and allows for biochemical identification of the species considering the specificity. Unfortunately, the slow doubling time of *M tuberculosis* makes culture on egg/ agar-based solid media slow and time consuming. Agar-based media allow detection of colonies in 10-12 days, whereas most commonly used Lowenstein Jensen Medium (LJ) usually takes 18-24 days.

Liquid Culture Methods

Bacteria TB 460 is a sensitive, specific and rapid culture method for smear positive respiratory as well as non-respiratory specimens. Time for detection of M tuberculosis complex from smear negative clinical specimen is 13-15 days. In specimens, which are difficult to obtain such as tissue biopsies and body fluids, the use of LJ media and BancTec TB 460 may be justified to maximize isolation of mycobacteria.

REFERENCES

1. Aaron, L, Saadoun D, Calatroni I. Tuberculosis in HIV-infected patients: a comprehensive review. Clinical Microbiology and Infection, 2004; 10: 388-398.
2. Abdallah, AM, Verboom T, Hannes F. A specific secretion system mediates PPE41 transport in pathogenic mycobacteria. Mol Microbiol., 2006; 62: 667-679.
3. Abdallah AM, Verboom T, Weerdenburg EM. PPE and PE_PGRS proteins of Mycobacterium marinum are transported via the type VII secretion system ESX-5. Mol Microbiol., 2009; 73: 329-340.
4. Abramovitch, R. B., Rohde, K. H., Hsu, F.-F. & Russell, D. G., Apr ABC: Mycobacterium tuberculosis complex-specific locus that modulates pH-drivenadaptation to the macrophage phagosome. Molecular Microbiology, 2011; 80: 678-694.
5. Adams, Kristin N., Takaki, K., Connolly, Lynn E., Wiedenhoft, H., Winglee, K., Humbert, O., Edelstein, Paul H., Cosma, Christine L. & Ramakrishnan, L. Drug Tolerance in Replicating Mycobacteria Mediated by a Macrophage-Induced Efflux Mechanism. Cell, 2011; 145: 39-53.
6. Adler, J. J., Rose, D. N. Transmission and pathogenesis of tuberculosis. In Tuberculosis, Edited by W. N. Rom & S. M. Garay: Boston: Little, Brown, 1996, pp. 129-140.

7. Agerberth, B. & Guðmundsson, G. H. Host Antimicrobial Defence Peptides in Human Disease. In Antimicrobial Peptides and Human Disease, Edited by W. Shafer: Springer Berlin Heidelberg, 2006, pp. 67-90.
8. Alcaïs, A., Fieschi, C., Abel, L. & Casanova, J.-L. Tuberculosis in children and adults: two distinct genetic diseases. The Journal of Experimental Medicine, 2005; 202: 1617-1621.
9. Alcaïs, A., Quintana-Murci, L., Thaler, D. S., Schurr, E., Abel, L. & Casanova, J.-L. Life-threatening infectious diseases of childhood: single-gene inborn errors of immunity? Annals of the New York Academy of Sciences, 2010; 1214: 18-33.
10. Alonso, S., Pethe, K., Russell, D. G. & Purdy, G. E. Lysosomal killing of Mycobacterium mediated by ubiquitin-derived peptides is enhanced by autophagy. Proc Natl Acad Sci U S A, 2007; 104: 6031-6036.
11. Andrews, J. R., Noubary, F., Walensky, R. P., Cerda, R., Losina, E. & Horsburgh, C. R. Risk of progression to active tuberculosis following reinfection with Mycobacterium tuberculosis. Clin Infect Dis., 2012; 54: 784-791.
12. Arnvig, K. B., Comas, I., Thomson, N. R. Sequence- Based Analysis Uncovers an Abundance of Non-Coding RNA in the Total Transcriptome of Mycobacterium tuberculosis. PLoS Pathog., 2011; 7: e1002342.
13. Aronson, J. D. & Whitney, C. E. The Types of Tubercle Bacilli Found in Tuberculous Lesions and in Nontuberculous Tissue in Man. Journal of Infectious Diseases, 1930; 47: 30-55.
14. Arya, S., Sethi, D., Singh, S. Truncated Hemoglobin, HbN, Is Post-translationally Modified in Mycobacterium tuberculosis and Modulates Host-Pathogen Interactions during Intracellular Infection. Journal of Biological Chemistry, 2013; 288: 29987-29999.
15. Asensio, J. A., Arbues, A., Perez, E., Gicquel, B., Martin, C. Live tuberculosis vaccines based on phoP mutants: a step towards clinical trials. Expert Opin Biol Ther., 2008; 8: 201-211.
16. Awasthy, D., Gaonkar, S., Shandil, R. K., Yadav, R., Bharath, S., Marcel, N., Subbulakshmi, V. & Sharma, U. Inactivation of the ilvB1 gene in Mycobacterium tuberculosis leads to branched-chain amino acid auxotrophy and attenuation of virulence in mice. Microbiology, 2009a; 155: 2978-2987.
17. Bach, H., Papavinasasundaram, K. G., Wong, D., Hmama, Z. & Av-Gay, Y. Mycobacterium tuberculosis virulence is mediated by PtpA dephosphorylation of human vacuolar protein sorting 33B. Cell Host Microbe, 2008; 3: 316-322.
18. Balganesh, M., Dinesh, N., Sharma, S., Kuruppath, S., Nair, A. V. & Sharma, U. Efflux Pumps of Mycobacterium tuberculosis Play a Significant Role in Antituberculosis Activity of Potential Drug Candidates. Antimicrobial Agents and Chemotherapy, 2012; 56: 2643-2651.
19. Bardarov, S., Bardarov Jr, S., Jr., Pavelka Jr, M. S., Jr., Sambandamurthy, V., Larsen, M., Tufariello, J., Chan, J., Hatfull, G. & Jacobs Jr, W. R., Jr. Specialized transduction: an efficient method for generating marked and unmarked targeted gene disruptions in Mycobacterium tuberculosis, M. bovis BCG and M. smegmatis. Microbiology, 2002; 148: 3007-3017.
20. Barnes, P. F., Leedom, J. M., Chan, L. S., Wong, S. F., Shah, J., Vachon, L. A., Overturf, G. D. & Modlin, R. L. Predictors of Short-Term Prognosis in Patients with Pulmonary Tuberculosis. Journal of Infectious Diseases, 1988; 158: 366- 371.
21. Bloom, B. R., and C. J. L. Murray. Tuberculosis: commentary on areemergent killer. Science, 1992; 257: 1055–1064.
22. Kent, L., T. D. McHugh, O. Billington, J. W. Dale, and S. H. Gillespie. Demonstration of homology between IS6110 of Mycobacterium tuberculosis and DNAs of other Mycobacterium spp.? J. Clin. Microbiol., 1995; 33: 2290–2293.
23. Kim, B. J., K. H. Lee, B. N. Park, S. J. Kim, E. M. Park, Y. G. Park, G. H. Bai, S. J. Kim, and Y. H. Kook. Detection of rifampin-resistant Mycobacterium tuberculosis in sputa by nested PCR-

linked single-strand conformation polymorphism and DNA sequencing. J. Clin. Microbiol. 2001; 39: 2610– 2617.
24. F. C. Bange, and E. C. Bottger. Genotypic identification of mycobacterial by nucleic acid sequence determination: report of a 2-year experience in a clinical laboratory. J. Clin. Microbiol. 1993; 31: 2882–2889.
25. Telenti, A., P. Imboden, F. Marchesi, D. Lowrie, S. Cole, M. J. Colston, L. Matter, K. Schopfer, and T. Bodmer. Detection of rifampin-resistance mutations in Mycobacterium tuberculosis. Lancet, 1993; 341: 647–650.
26. Weil, A., B. B. Plikaytis, W. R. Butler, C. L. Woodley, and T. M. Shinnick. The mtp40 gene is not present in all strains of Mycobacterium tuberculosis. J. Clin. Microbiol., 1996; 34: 2309–2311.
27. Wolinsky, E. Mycobacterial diseases other than tuberculosis. Clin. Infect. Dis., 1992; 15: 1–10.
28. Yuen LK, BC Ross, Jackson KM, and Dwyer B. Characterization of Mycobacterium tuberculosis strains from Vietnamese patients by Southern blot hybridization. J. Clin. Microbiol., 1993; 31: 1615–1618.

How to cite this article: Nowshad Z, Shrivastava V, Mathur A et al. Multi drug resistant tuberculosis: an emerging disease in today's scenario of Kashmir (J&K), India. International Journal of Research and Review. 2017; 4(5):161-180.

******