**Molecular Revealing of Non-Tuberculous Mycobacterium-Clinical Usage to Diagnose Hidden Danger**

**Abstract:** Significantly it is important to realize that there is no "stand alone" assay for the identification of Non-Tuberculous Mycobacterium (NTM). Newer molecular assays are more defined for the detection than phenotypic tests. Study includes molecular differentiation of Mycobacterium tuberculosis complex (MTC) from Mycobacterium other than Tuberculosis (MOTT). Present study was carried out for the differentiation of Mycobacterium tuberculosis complex and Non tuberculous mycobacteria utilizing rpoB gene as a molecular marker.

**Keywords:** Amplimer, Genitourinary tract, Heat shock protein, Petroffe’s method, Nontuberculous mycobacteria, Lymphadenitis

**Introduction**

Nontuberculous mycobacteria (NTM) have been known since the time of Robert Koch, but historically they have been overshadowed by tuberculosis and dismissed as contaminants. (1). NTM have been variously described by the adjectives as “atypical”, “anonymous”, mycobacteria other than TB (MOTT), “environmental”, “environmental opportunistic”, and, seemingly most commonly in the medical literature, “non tuberculous” or “NTM” (2, 3). More than 125 species of NTM have been identified, approximately 60 of which are suspected or known to be pathogenic. There are numerous clinical complications associated with NTM (4). Of the 132 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 (5). 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. 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 tract (6, 7). *M. avium* or *M. kansasii* infection are the predominant species known in AIDS patients, but nowadays *M. avium* is frequently encountered in immunocompetent patients, probably due to the improved diagnostic tools and clinical awareness (8-10). Cutaneous MOTT infections result from external inoculation at sites of trauma, the spread of a deeper infection from the joints or other tissues, or haematogenous spread of a disseminated infection (11). MOTT lymphadenitis is seen in immunocompromised patients, but is mostly known as the most common manifestation of MOTT disease in immunocompetent patients and usually affects children under the age of 12 as chronic cervicofacial lymphadenitis (12-17). Diagnosis includes, culture, biochemical characterization etc. Molecular techniques have replaced conventional methods for appropriate species identification (18). Numerous genetic targets have been described for the species differentiation. Some of them includes; the partial genes for 65 kDa heat shock protein (*hsp65*), RNA polymerase beta-subunit (*rpoB*), essential protein *secA1*, superoxide dismutase (*sodA*), 16S RNA and the 16S–23S ribosomal RNA internal transcribed spacer (ITS) (19). Thus the present study was carried out for the differentiation of *Mycobacterium Tuberculosis* complex (MTC) and MOTT, targeting two different sets of primers *rpoB* gene.

**Materials and Methods:**

38 different specimens which includes; urine, pus, lymph nodes, mucosal biopsy, CSF, sputum, pleural fluid (from patients suspected for tuberculosis were considered) from different departments of SMI Hospital, Patel Nagar, Dehradun. The study was approved by Institutional Ethical Committee. The specimens were further processed for AFB smears, Tuberculosis PCR targeting *rpoB* gene. Viscous specimens were decontaminated by modified petroffe’s method. Decontamination solution was prepared by adding 0.5 % NALC (w/v; Sigma) to a 1:1 mixture of 4% NaOH and 2.9% sodium citrate. Tissue specimens were grinded in a screw cap tube by adding silica beads in a tissue grinder. The specimens were centrifuged in a 15 ml or 50 ml sterile centrifuge tubes at 5000 rpm for 20 minutes. Supernatant was discarded gently and the sediment was taken for PCR as well as for AFB smears preparation. 200 µl of the sediment was taken for PCR. DNA was extracted with nucleopore DNA extraction kit from all the specimens utilizing silica columns (20).

**Primers Selection:** Mycobacterium tuberculosis and members of MTC naturally contains a defective oxyR due to mutations at 5’-end of the gene but NTM have a functional gene copy for the same. *rpoB* gene among mycobacterial species contains variable sequences and can be utilized for identification and differentiation of MTC from NTM. MTC specific primers sequences at 3’-end contains an additional nucleotide variable *rpoB* gene region from MTC and NTM can be amplified using MTC-specific primers with sequence; [MTCF, 5’-TACGGTCGCGACGCTGATCCAAA-3’] and MTCR, 5’-ACAGTCGCGCTTGTGGGTCAAC-3’] or NTM-specific primers [NTMF, 5’-GGAGCGGATGACCACCCAGGACGTC-3’] and NTMR, 5’- CAGCGGGTTGTCTGTCCATGAAC-3’] which is yielding amplicons of 235 bp and 136bp.
for MTC and NTM respectively (20). Briefly, for the single reaction mixture in a final volume of 25µl containing buffer (10X)-10µl; dNTPs (100mM)-0.4µl; primers (MTCF, MTCR, NTMF, NTMR) [25µM]-1µl each; Taq DNA polymerase (2U/µL)-1µl; nuclease free water and template DNA. The cycling parameters include: an initial denaturation at 94ºC for 5 minutes; 40 repetitive cycles at 94ºC for 30 seconds, 64ºC for 1 minute, 72ºC for 1 minute; final extension at 72ºC for 5 minutes. The amplified products were analyzed by gel electrophoresis in 1.4% agarose gel.

Discussion and Conclusion:

NTM are a varied group of mycobacterial species, causing a range of human diseases, being ubiquitously, they are present in the environment and can therefore be associated a range of clinical diseases. NTM have the potential to cause diseases in both immuno-compromised and immunocompetent individuals, other diseases caused by NTM includes; lymphadenitis, pulmonary infections, disseminated disease. The role of NTM in human disease in may well be underestimated and should be examined in more detail and on a larger scale. Information is immediately required for the proper diagnostic procedures and the possibilities for adequate treatment of NTM induced disease. Because of differences in antimicrobial susceptibility that determine treatment options, species-level identification of the NTM is becoming increasingly clinically important (21-24). The diagnosis of NTM disease is not simple. Nucleic acid amplification technologies utilization in molecular diagnostic methods can gradually replace traditional assays for NTM identification. Significant approach of utilizing a single assay in Mycobacteriology laboratory for the detection and differentiation of MTC and MOTT can be very useful (25). Being a novel, rapid, sensitive and specific technology, the same can be utilized for the epidemiological surveys of tuberculosis infections as well as to diagnose the hidden danger. Present study is very useful for the differentiation of MTC from NTM, which can be further very significant for the further treatment and disease management.

Lane 1: Well 1= Negative specimen for NTM and MTC.
Well 2, 4, 6= Positive for MTC.
Well 3= positive for NTM.
Well 7= Positive control.
Well 8= 100bp DNA difference ladder.

Results:

Out of 38 specimens processed, amplimer of 235bp which is specific for MTC was observed in 6 specimens, where as 5 cases showed an amplicon of 136bp specific for NTM. Two pus specimens showed mixed infection for MTC and NTM yielding an amplicon size of 235bp and 136bp respectively.
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References:


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