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

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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

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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 characterizitation 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 (*rpo*B), essential protein *sec*A1, superoxide dismutase (*sod*A), 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'-TACGGTCGGCGAGCTGATCCAAA-3' and MTCR, 5'-ACAGTCGGCGCTTGTGGGTCAAC-3'] or NTM-specific primers [NTMF, 5'-GGAGCGGATGACCACCCAGGACGTC-3' and NTMR, 5'- CAGCGGGTTGTTCTGGTCCATGAAC-3'] which is yielding amplicons of 235 bp and 136bp

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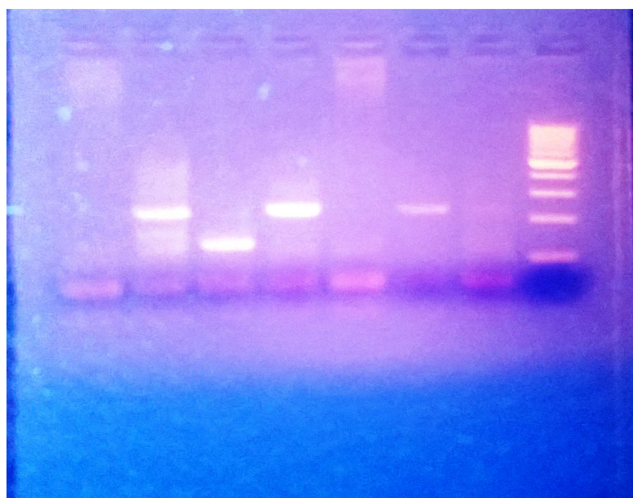
for MTC and NTM respectively (20). Briefly, for the single reaction mixture in a final volume of 25µl containing buffer (10X)-10µl;dNTPs(100milliM) 0.4µl;primers(MTCF,MTCR,NTMF,NTMR) [25µM]-1µl each; Taq DNA polymerase(2U/µL)-1µl; nuclease free water and template DNA.The cycling parameters includes: an initial denaturation at 94°C for 5 minutes; 40 repetitive cycles at 94°C for 30 seconds, 64°C for 1 minute, 72°C for 1 minute; final extension at 72°C for 5 minutes. The amplified products were analyzed by gel electrophoresis in 1.4% agarose gel.



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.

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.

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Conflict of Interest: None

References:

1) American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am Rev Respir Dis 1990;142:940–953.
2) Tortoli E. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990's. Clin Microbiol Rev 2003;2:319–354.
3) Falkinham JO. Nontuberculous mycobacteria in the environment. Clin Chest Med 2002;23:520–551.
4) O'Brien RJ, Geiter LJ, Snider DE. The epidemiology of nontuberculous mycobacterial diseases in the United States: results from a national survey. Am Rev Respir Dis 1987;135:1007–1014.
5) Horsburgh CR Jr. Epidemiology of Mycobacterium avium complex. In: Korvick JA, Benson CA, editors. Mycobacterium avium complex infection: progress in research and treatment. New York: Marcel Dekker; 1996. pp. 1–22.
6) Centers for Disease Control and Prevention. Nontuberculous mycobacteria reported to the public health laboratory information system by state public health laboratories: United States, 1993–1996. cdc.gov/ ncidod/dastlr/mycobacteriology.htm (no longer available) (accessed July 1999).
7) Horsburgh CR Jr. Epidemiology of disease caused by nontuberculous mycobacteria. Semin Respir Infect 1996; 11:244.
8) Olivier KN, Weber DJ, Lee JH, Handler A, Tudor G, Molina PL, Tomashefski J, Knowles MR; Nontuberculous Mycobacteria in Cystic Fibrosis Study Group. Non-tuberculous mycobacteria. II: nestedcohort study of impact on cystic fibrosis lung disease. Am J Respir Crit Care Med 2003;167:835–840.
9) Witty LA, Tapson VF, Piantadosi CA. Isolation of mycobacteria in patients with pulmonary alveolar proteinosis. Medicine (Baltimore) 1994;73:103.
10) Hadjiliadis D, Adlakha A, Prakash UB. Rapidly growing mycobacterial lung infection in association with esophageal disorders. Mayo Clin Proc 1999;74:45.
11) Griffith DE, Girard WM, Wallace RJ Jr. Clinical features of pulmonary disease caused by rapidly growing mycobacteria: an analysis of 154 patients. Am Rev Respir Dis 1993;147:1271.
12) Huang JH, Kao PN, Adi V, Ruoss SJ. Mycobacterium avium-intracellulare pulmonary infection in HIV-negative patients without pre-existing lung disease: diagnostic and management limitations. Chest 1999;115:1033–1040.
13) Wallace RJ Jr, Zhang Y, Brown BA, Dawson D, Murphy DT, Wilson R, Griffith DE. Polyclonal Mycobacterium avium complex infections in patients with nodular bronchiectasis. Am J Respir Crit Care Med 1998; 158:1235–1244.
14) Keane J, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001; 345:1098–1104.
15) Keane J. Tumor necrosis factor blockers and reactivation of latent tuberculosis. Clin Infect Dis 2004; 39:300–302.
16) Wallis RS, Broder MS, Wong JY, Hanson ME, Beenhouwer DO. Granulomatous infectious diseases associated with tumor necrosis factor antagonists. Clin Infect Dis 2004; 38:1261–1265.

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17) National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Twelfth Informational Supplement. Wayne, PA: NCCLS; 2002. Document No. M100-S12.

18) Gingeras, T. R., G. Ghandour, E. Wang, A. Berno, P. M. Small, F. Drobniewski, et al. 1998. Simultaneous genotyping and species identification using hybridization pattern recognition analysis of generic Mycobacterium DNA arrays. Genome Res. 8:435–448.

19) Goto, M., S. Oka, K. Okuzumi, S. Kimura, and K. Shimada. 1991. Evaluation of acridinium-ester-labeled DNA probes for identification of Mycobacterium tuberculosis and Mycobacterium avium-Mycobacterium intracellulare complex in culture. J. Clin. Microbiol. 29:2473–2476.

20) Mokaddas, Eiman, and Suhail Ahmad. "Development and evaluation of a multiplex PCR for rapid detection and differentiation of Mycobacterium tuberculosis complex members from non-tuberculous mycobacteria." Japanese journal of infectious diseases 60.2/3 (2007): 140.

21) National Committee for Clinical Laboratory Standards. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes. Approved Standard. Wayne, PA: NCCLS; 2003. Document No. M24-A.

22) Sharma, Narotam, Amitabh Talwar, Satish C. Nautiyal, Rakhi Kaushik, Amanpreet Singh, Akhil Pratap Singh, Jivesh K. Shaw, Shilpi Dixit, and R. K. Singh. "Nested-PCR and Uracil-N-Glycosylase-significant approach to prevent amplicon contamination in tuberculosis PCR performing laboratories." Archives of Applied Science Research 5, no. 2 (2013): 177-179.

23) Sharma N, Sharma V, Singh PR, Jawed B, Babu V, Kandpal J, et al. Tuberculosis and Molecular Diagnosis. WebmedCentral BIOTECHNOLOGY 2013;4(2):WMC003992

24) Sharma, Narotam, Veena Sharma, Prem Raj Singh, Shivani Sailwal, Rajeev S. Kushwaha, Rajesh K. Singh, Satish C. Nautiyal, Pankaj Mishra, Tariq Masood, and R. K. Singh. "Diagnostic Value of PCR in Genitourinary Tuberculosis." *Indian Journal of Clinical Biochemistry*: 1-4.

25) Sharma Narotam, Sharma Veena, Nautiyal Satish Chandra, Singh Prem Raj, Rajeev S, Kushwaha, Sailwal Shivani, Ghosh Shayan, Naushad Ahmer, Singh R.K. Conventional PCR usage for the detection of Mycobacterium Tuberculosis complex in Cerebrospinal Fluid by MPB64-Target PCR. *International Journal of Drug Development & Research, October-December 201, Vol. 4, Issue 4, ISSN 0975-9344.*

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