Case Report

Recovery of *Mycobacterium lentiflavum* from Bronchial Lavage during Follow-up of an Extrapulmonary Tuberculosis Patient

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Abstract

Initially diagnosed with cervical lymphadenitis, a 15-year-old boy was started with category I anti-tuberculosis (TB) drugs. Follow-up investigations led to isolation and identification of *Mycobacterium lentiflavum* by multiple diagnostic and identification approaches. Observation of this rare pathogen from human origin urges cautious diagnosis while attending TB cases.

**Keywords:** Extrapulmonary tuberculosis, India, lymphadenitis, *Mycobacterium lentiflavum*, tuberculosis

Introduction

*Mycobacterium lentiflavum*, a slow-growing, pigmented nontuberculous *Mycobacterium*, is a rare entity of human disease. Thomas *et al.*, on analyzing 48 cases of *M. lentiflavum* infection observed lung as a common site of infection in adults and cervical lymphadenitis to be a common manifestation in minors.\(^1\) Similar manifestations in pediatric cases by *M. lentiflavum* with poor treatment outcome were also reported.\(^2,3\) Nontuberculous mycobacterial infections are of therapeutic challenge as clinical presentation is often indistinguishable from tuberculosis (TB), and species-specific treatment scheme is necessary. Reviewing the currently recommended guidelines on the aspects of mycobacterial treatment, Esteban *et al.* also highlighted the difficulty to establish proper guidelines for rare pathogenic species.\(^4\) Although *Mycobacterium fortuitum*, *Mycobacterium avium* complex, *Mycobacterium abscessus*, and *Mycobacterium chelonae* remain the common clinical isolations of nontuberculous mycobacteria (NTM) in Asia and Middle-East countries,\(^5,6\) the distribution of NTM is diverse even within a country. With rampant increase in NTM isolations, careful discernment of clinical significance and colonization or contamination is important as environment plays a key role in harboring these entities.\(^7\) Water-related nosocomial outbreak by *M. abscessus* in a pediatric surgical ward has been reported from India.\(^8\) In this study, we report the isolation of *M. lentiflavum* from bronchial lavage of a boy associated with cervical lymphadenitis and lung consolidation.

Case Report

A 15-year-old boy reported to a government block-level dispensary with scrotal inflammation and pain. The clinical symptoms included weight loss, lymphadenitis, intermittent fever, ascites, and hydrocele. He was clinically suspected to be suffering from extrapulmonary TB-cervical Koch’s lymphadenitis. Further tests showed positive Mantoux test, acid-fast bacilli in cervical lymph node biopsy, and a negative sputum smear. Erythrocyte sedimentation rate (ESR) was mildly raised (20 mm/h), lung X-ray presented prominent mediastinal lymph nodes, and lower abdominal ultrasound revealed moderate ascites with few subcentimeter mesenteric lymph node in the preumblical region. The patient was started on category I anti-TB drugs, and the HIV status is unknown. Though advised an operative procedure for hydrocele, the

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procedure could not be performed for economic reasons. Given the mother’s health history of succumbing to TB, the patient was closely examined for TB while reporting for scrotal pain and inflammation.

The follow-up laboratory test results at the end of 6-month treatment course are as follows: ESR elevated (34 mm/h), alkaline phosphatase on the upper limit (202 U/L), and left pleural effusion with the underlying consolidation in lung X-ray. The ascites and cervical lymph node swelling had subsided. The case was then referred to a tertiary care hospital acquiring a Revised National Tuberculosis Control Program unit with better-equipped identification facility for Mycobacterium species for further investigation. The child’s bronchial lavage was acid-fast smear negative but was flagged as positive for Mycobacterium species when inoculated in BACTEC MGIT™ 320 (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), with culture showing acid-fast report of 3+ grade. The isolate (PK504) was presumed as NTM on the basis of a negative MPT64 protein detection-based immunochromatographic test (SD MPT64TB Ag Kit, Standard Diagnostics, Inc., Gyeonggi-do, South Korea). This led to change of treatment to NTM treatment regimen comprising rifampicin (300 mg/day), isoniazid (300 mg/day), ethambutol (600 mg/day), and clarithromycin (500 mg/day). In between these two mycobacterial treatments, he had been prescribed two short courses of conventional antibiotics.

The isolate was shared with our laboratory and species was confirmed to be M. lentiflavum with the help of culture growth [Figure 1] and molecular tests. An informed consent was obtained from the patient after clear understanding of the study purpose. Biochemical tests such was niacin production, nitrate reductase, catalase, and pyrazinamidase were also performed. The isolate was positive for catalase (heat stable and semiquantitative) and pyrazinamidase but negative for niacin production and nitrate reductase. For molecular identification, DNA was extracted by DNA isolation kit (Qiagen Inc., Valencia, USA) and used in polymerase chain reaction (PCR) amplification for Mycobacterium genus-specific hsp65 gene which amplified a partial 441 bp segment, confirming it to be of Mycobacterium genus.[9] The species-specific PCR for Mycobacterium tuberculosis and Mycobacterium bovis was also tested with RD9, RD4, and 500 bp primers which were all negative for M. tuberculosis complex, suggesting the isolate to be NTM. PCR-restriction enzyme pattern analysis (PRA) was performed on the purified product of hsp65 amplicon using the enzymes BstEII and HaeIII (New England Biolabs Inc., USA) to identify the species.[9] The band fragmentation pattern (BstEII-no digestion; HaeIII-145/130 bp) directed the isolate to three NTM species, namely, M. lentiflavum, Mycobacterium florentinum, and Mycobacterium simiae, as per the published algorithm of PRA-hsp65 patterns.[10] In addition, the purified PCR product of hsp65 was also sequenced and submitted to GenBank, National Center for Biotechnology Information (NCBI) (KX431212). The isolate’s hsp65 sequence was used for blast analysis in the NCBI for identification which depicted 100% match to M. lentiflavum. Phylogenetic analysis was also carried out based on the sequenced hsp65 gene of the isolate along with the reference strain of M. lentiflavum (AF547851), other closely related species (GQ153292, JF491317), the best matches of BLAST results (KF432468, KF432466, KF432457), and two out-groups (AY299144, AF547813). All sequences were aligned using ClustalW program in the freely available online software tool MEGA 6 (www.megasoftware.net/), and the dendrogram was inferred using the maximum likelihood approach [Figure 2]. The phylogenetic tree depicted the isolate (PK504) to be M. lentiflavum, forming a clad with reference and clinical Chinese strains of M. lentiflavum. Following gene sequence identification, the isolate was also identified with MALDI-TOF technology given its significance as a clinical diagnostic tool. Using freshly grown culture, the isolate was processed according to the manufacturer’s protocol with Escherichia coli ATCC 8739 as control. Employing the

![Figure 1: Smooth, scotochromogenic colonies observed on Lowenstein–Jensen medium (glycerol)](image)
SARAMIS Research Use Only database in MALDI-TOF Vitek MS system (BioMerieux, France), the isolate was identified as *M. lentiflavum* with a percent probability value of 86.9%. After species confirmation, the same NTM therapeutic regimen was continued as the boy responded positively to the therapy. The patient is cured of lymphadenitis, lung consolidation, and ascites. Regaining vigor, he is able to pursue academics and perform daily activities.

**Discussion**

The study accounts the isolation of *M. lentiflavum* from a minor suffering from Koch’s lymphadenitis or scrofula who had already completed 6-month course of category I anti-TB drug regimen. The relapse cases of TB are either drug-resistant TB or NTM infections. Such cases in resource-constrained settings are very often misdiagnosed or sometimes given appropriate attention only after obtaining a history of failed anti-TB treatments. Rapid, affordable, and differential diagnostics adjunct to collaborative efforts will effectively mitigate such episodes. *M. lentiflavum* isolated in this study depicts the presence of this rarely reported pathogen in the country which cannot be overlooked as it may represent many such unreported cases. The number of NTM cases has not only been increasing in the region but rare NTM species are also being reported which makes clinical diagnosis and treatment challenging, especially in lesser developed settings.

This case of *M. lentiflavum* isolated from a child with lymphadenitis is possibly only the second report of *M. lentiflavum* isolation from human source and typical of its kind from child TB cases in India. The first isolation in the country was communicated from All India Institute of Medical Sciences, New Delhi, with a GenBank sequence submission of *M. lentiflavum* 16S rRNA gene (JQ037846). Isolation of this NTM species illustrates the clinical relevance of NTM infections in susceptible individuals and calls for cautious diagnosis and treatment while managing TB cases in this TB-laden country.

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**Conflicts of interest**

There are no conflicts of interest.

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