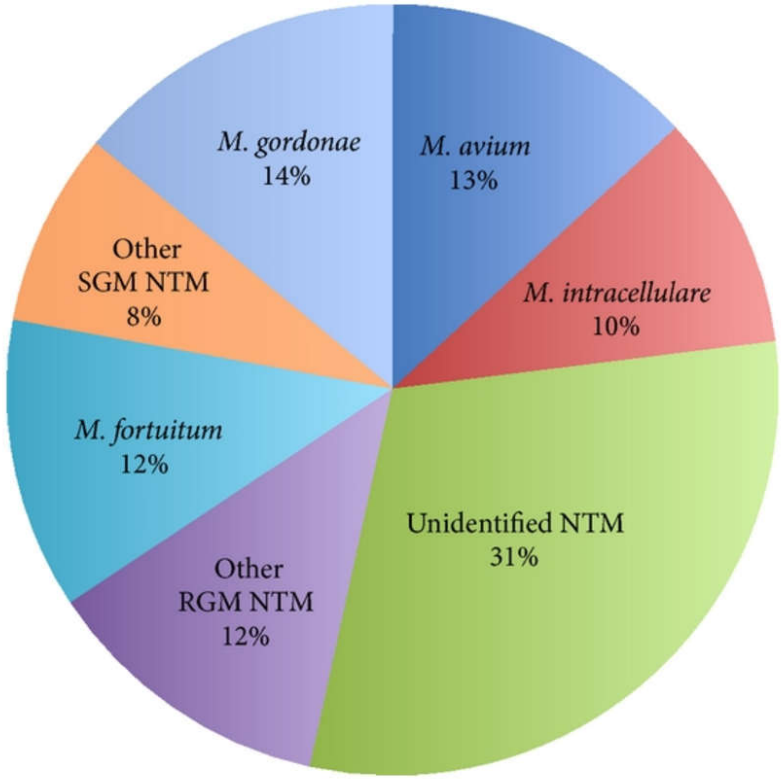
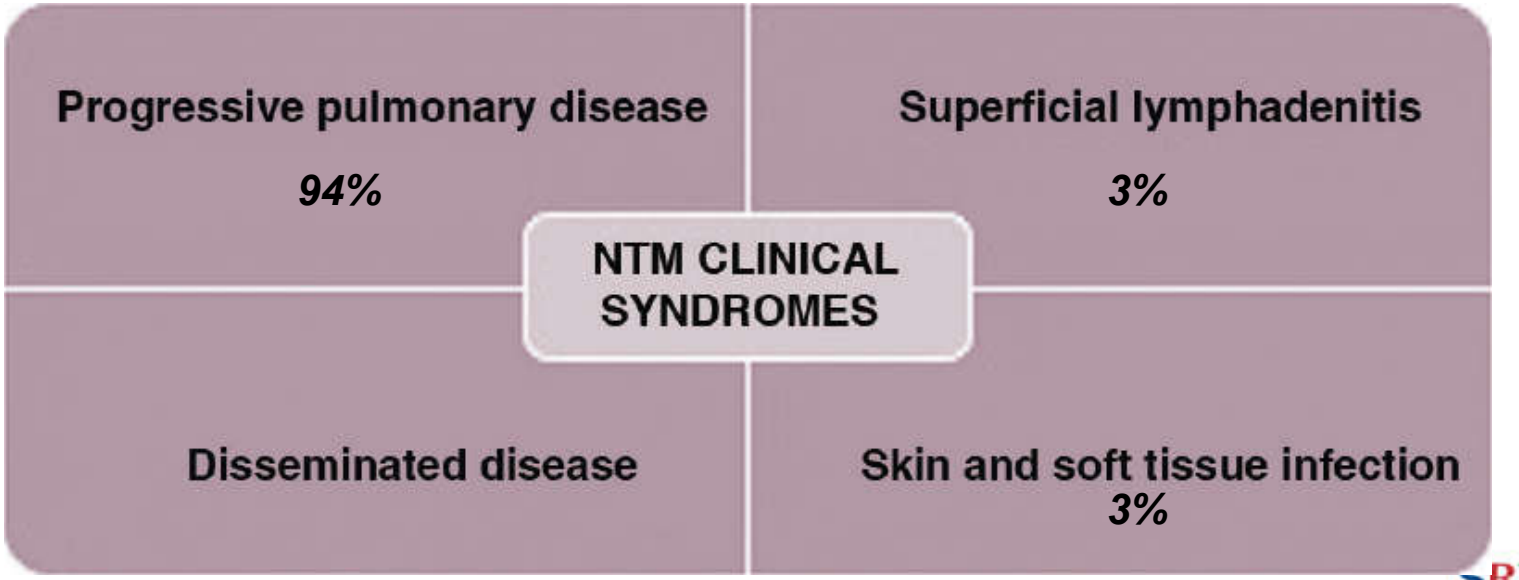
Nontuberculous Mycobacteria Diagnostics



Medimail
Dec 2018

Nontuberculous Mycobacteria (NTM)

- Aka mycobacteria other than tubercle bacilli (MOTT) OR atypical OR environmental mycobacteria
- Recognized as a cause of human disease since 1950s
- Ever-expanding list of isolation of organisms in genus *Mycobacterium*



Clinical Syndromes of NTM Infection in Humans

Classification of NTM

Slow growers			Runyon IV: Rapid growers
Runyon I: Photochromogens	**Runyon II: Scotochromogen**	**Runyon III: Nonchromogens**	
Slow growing; produce yellow orange pigment when exposed to light	Slow growing; produce yellow orange pigment in dark	Slow growing; no pigment production when exposed to light	Rapid growing; They do not produce pigment or are late pigmenters
M. kansasii, M. marinum, M. asiaticum, and M. simiae	*M. gordonae and M. scrofulaceum*	*M. avium and M. intracellulare (M. avium-intracellulare complex), M. ulcerans*	*M. fortuitum, M. peregrinum, M. abscessus, M. chelonae, M. thermoresistibile*

- Till 1990s, ~50 NTM species known
- With recent advances in molecular techniques, over 125 NTM species identified
- 16S rRNA gene sequencing is a standard for new species cataloguing
- However, for all classification purposes, NTM can be broadly classified into slow growers and rapid growers

Sharma S. Astrocyte 2017. 4. 67. 10.4103/astrocyte.astrocyte_54_17

Epidemiology

- NTM prevalence in India – 7.4 to 27.4%
- Isolation rate of NTM in India – 0.7% to 34%
- Isolation rate – 3.5% in seronegative patients
- In India, most common isolates –
  - *M. fortuitum*
  - *M. intracellulare*
- In United States, most common spp –
  - *M. avium* complex (MAC)
  - *M. kansasii*
  - *Slow growing spp – M. marinum, M. xenopi, M. simiae, M. malmoense,* and *M. ulcerans*
  - Rapidly growing spp (RGM) – *M. abscessus*, *M. fortuitum*, and *M. chelonae*

Species of nontubercular mycobacteria	Frequency (%)
M. fortuitum	17 (27.5%)
M. intracellulare	13 (20.9%)
M. abscessus	9 (14.6%)
M. chelonae	8 (12.9%)
M. avium complex	5 (8.1%)
M. kansasii	3 (4.8%)
M. interjectum	2 (3.2%)
M. gordonae	2 (3.2%)
Other NTM	3 (4.8%)

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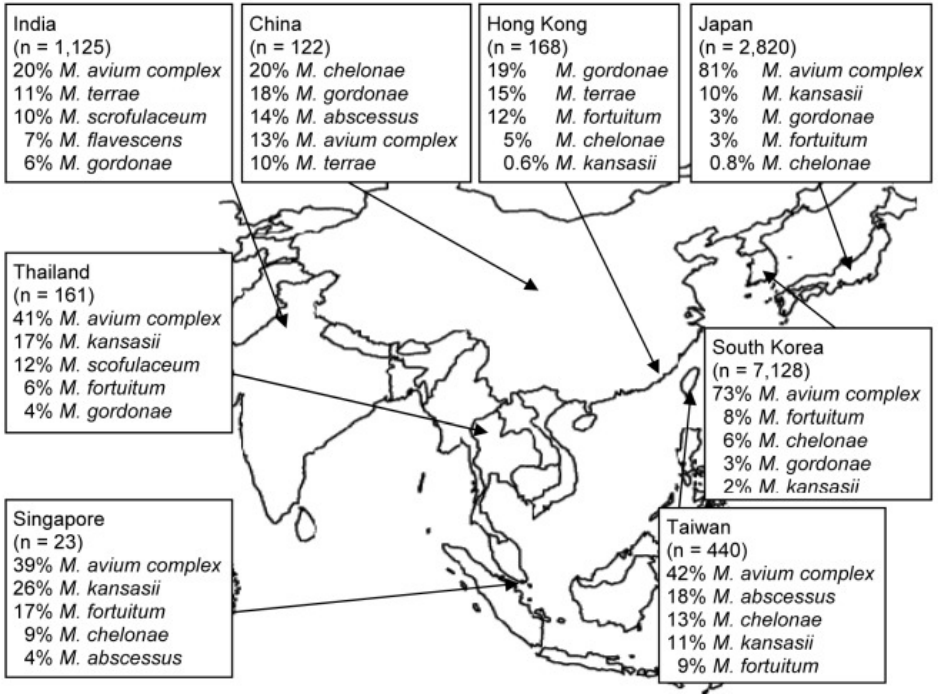
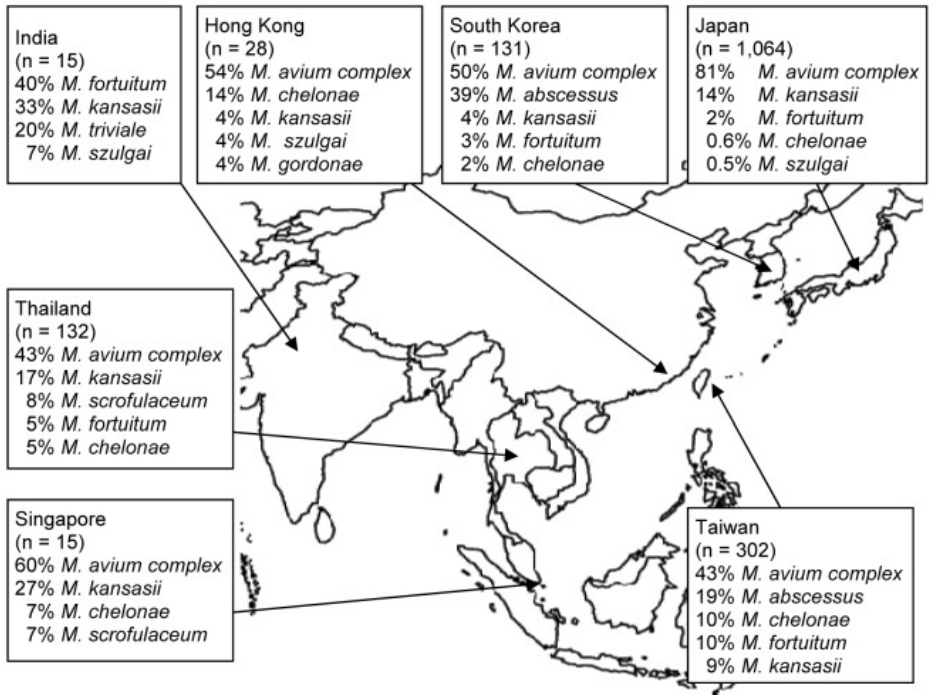
Maurya AK. Biomed Res Int. 2015;2015:465403

NTM Prevalence – Asia



5 most prevalent NTM spp found in respiratory specimens, regardless of clinical relevance, Asia, 1971–2007



5 most common NTM spp causing pulmonary infections, Asia, 1971–2007

Simons S. Emerg Infect Dis. 2011 Mar;17(3):343-9

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NTM Detection – Key Considerations

- Low index of clinical suspicion and following factors often result in delayed diagnosis –
  - Diagnosis of NTM lung disease requires considerable time due to its slow growth, and may be misdiagnosed as TB or other AFB-positive bacilli.
  - Symptoms are nonspecific like chronic cough, increased sputum production, dyspnea, low-grade fever, malaise, weight loss, & overlapping clinical characteristics with other lung diseases like bronchiectasis, COPD, bronchitis pulmonary TB.

- Chest radiography, sputum microscopy, and tuberculin skin testing are non-specific & fail to differentiate pulmonary tuberculosis from NTM infection. Hence, microbiologic confirmation for presence of NTM infection is recommended.

Joint Diagnostic Criteria – Clinical

American Thoracic Society (ATS) &
Infectious Disease Society of America (IDSA)

Pulmonary infection caused by NTM	
Fibrocavitary disease	**Fibronodular disease**
Male smokers	Elderly nonsmoking females
Underlying chronic pulmonary disease Lung upper lobes affected commonly	Almost always has bronchiectasis mostly affecting mid-lung fields

Features of Various Types of NTM Infections

AFB Smear and Culture

- AFB staining cannot differentiate between *M. tuberculosis* and NTM.

- Culture remains gold standard for laboratory confirmation of NTM and is required for genotypic identification and drug susceptibility tests (DST).

Diagnostic Criteria for NTM

Clinical (Both required)

1. Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high-resolution computed tomography scan that shows multifocal bronchiectasis with multiple small nodules

And

2. Appropriate exclusion of other diagnoses

Microbiologic

1. Positive culture results from at least two separate expectorated sputum samples. If the results from (1) are nondiagnostic, consider repeat sputum AFB smears and cultures

Or

2. Positive culture result from at least one bronchial wash or lavage

Or

3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM

Sharma S. Astrocyte 2017. 4. 67. 10.4103/astrocyte.astrocyte_54_17
Griffith DE. ATS/IDSA Statement. Am J Respir Crit Care Med. 2007 Feb 15; 175(4):367-416

Collection of Specimen

- Careful collection of high-quality respiratory specimens is necessary to avoid contamination since NTM are present in environment, especially in water sources.
- Temporary presence of NTM species from environmental sources in the airway may also lead to positive samples.
- Therefore, collection of three early-morning specimens on different days is preferred for diagnosis of NTM lung disease.

Ryu YJ. Tuberc Respir Dis (Seoul). 2016 Apr; 79(2): 74–84

Microbiological Outcomes

Culture Conversion: three consecutive negative mycobacterial sputum cultures collected over a minimum of 3 months, with the time of conversion being the date of the first of the three negative mycobacterial cultures. In patients unable to expectorate sputum, a single negative mycobacterial culture of a CT-directed bronchial wash is indicative of culture conversion.

Recurrence: two positive mycobacterial cultures following culture conversion. If available, genotyping may help distinguish relapse from reinfection.

Refractory Disease: failure to culture-convert after 12 months of non-tuberculous mycobacterial treatment.

Haworth CS, et al. Thorax 2017;72:ii1–ii64

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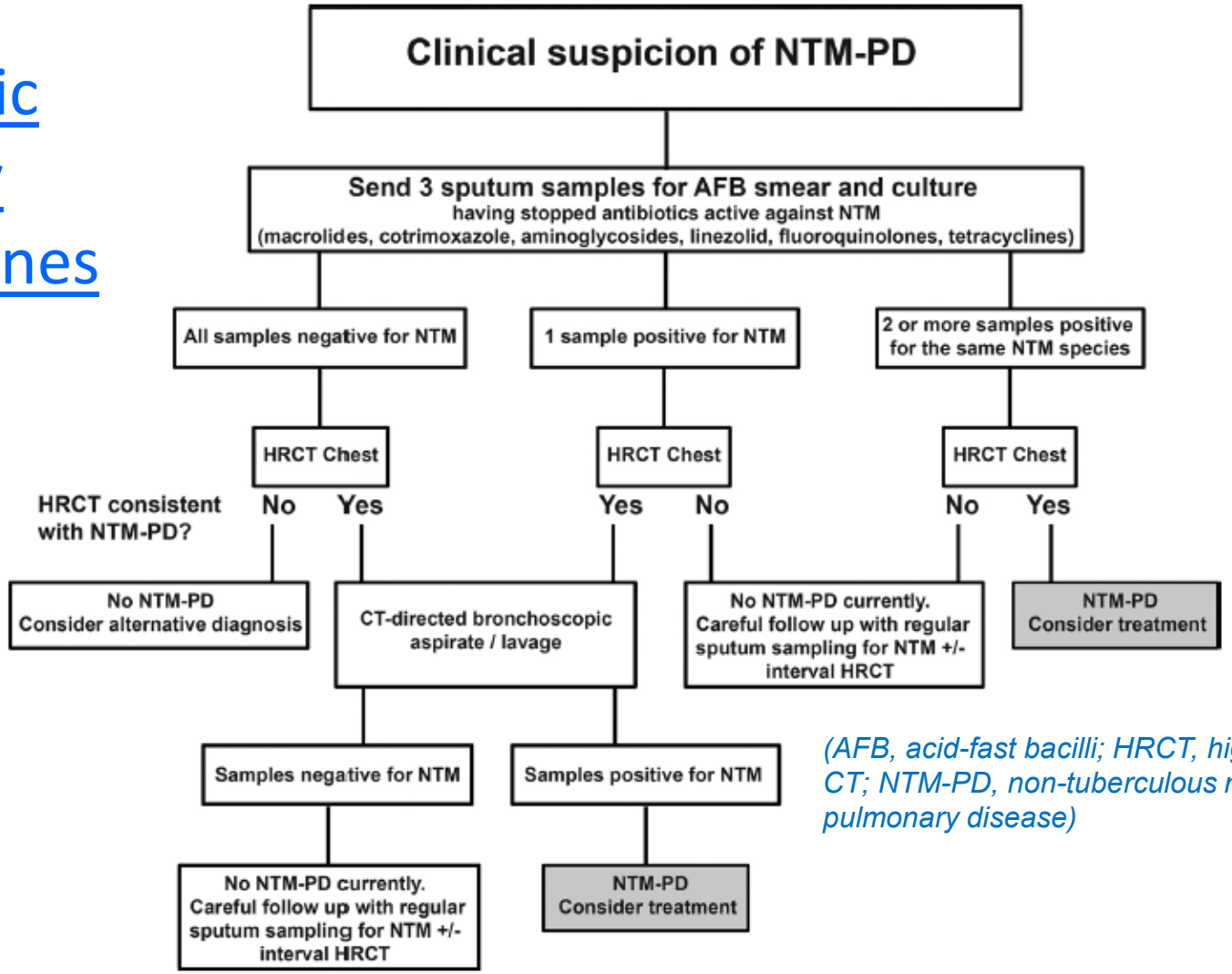
Algorithm for investigation of individuals with clinical suspicion of NTM-PD

British Thoracic Society Guidelines



(AFB, acid-fast bacilli; HRCT, high-resolution CT; NTM-PD, non-tuberculous mycobacterial pulmonary disease)

Haworth CS, et al. Thorax 2017;72:ii1–ii64

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Molecular Assays for NTM Identification/ Speciation

- Since treatments and outcomes differ depending on the NTM species, NTM identification is clinically important.
- Traditional biochemical tests (solid media, biochemical tests, and antimicrobial sensitivity testing) or HPLC for NTM identification need more time for detection and are less sensitive than molecular assays.
- So, they have been replaced by molecular methods such as line probe hybridization, polymerase chain reaction (PCR)-restriction restriction endonuclease assay (PRA), real-time PCR, and DNA sequencing.
- Gene sequencing is the reference method for the identification of NTM species and may be performed for uncommonly encountered species or precise identification at the subspecies level.

American Thoracic Society Documents

An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases

Am J Respir Crit Care Med Vol 175. pp 367–416, 2007
DOI: 10.1164/rccm.200604-571ST
Internet address: www.atsjournals.org

David E. Griffith, Timothy Aksamit, Barbara A. Brown-Elliott, Antonino Catanzaro, Charles Daley, Fred Gordin, Steven M. Holland, Robert Horsburgh, Gwen Huitt, Michael F. Iademarco, Michael Iseman, Kenneth Olivier, Stephen Ruoss, C. Fordham von Reyn, Richard J. Wallace, Jr., and Kevin Winthrop, on behalf of the ATS Mycobacterial Diseases Subcommittee

Recommendations

1. *Clinically significant NTM isolates should be routinely identified to the species level. An important exception is MAC because the differentiation between M. avium and M. intracellulare is not yet clinically significant. Although not routinely recommended, this differentiation may be important epidemiologically and, in the future, therapeutically (C, III).*
2. *The RGM (especially M. chelonae, M. abscessus, and M. fortuitum) should be identified to species level using a recognized acceptable methodology, such as PRA or biochemical testing, not HPLC alone (A, II).*
3. *Susceptibility of RGM for eight agents, including amikacin, cefoxitin, clarithromycin, ciprofloxacin, doxycycline, linezolid, sulfamethoxazole, and tobramycin, can also be used to facilitate identification of M. abscessus, M. chelonae, and M. fortuitum (C, III).*
4. *Communication between the clinician and laboratorian is essential for determining the importance and extent of the identification analysis for a clinical NTM isolate (C, III).*

Pre-treatment Drug Susceptibility Testing (DST)

- Used to guide the design of optimal treatment regimens.
- Role of DST is controversial because *in-vitro* susceptibility might not reflect *in-vivo* outcome, with the exception of macrolides and amikacin.
- Routine susceptibility testing of MAC isolates is recommended for clarithromycin only.
- Routine susceptibility testing of *M. kansasii* isolates is recommended for rifampin only.
- Routine susceptibility testing, for both taxonomic identification and treatment of RGM (*M. fortuitum*, *M abscessus*, and *M. chelonae*) should be with amikacin, imipenem (*M. fortuitum* only), doxycycline, the fluorinated quinolones, a sulfonamide or trimethoprim-sulfamethoxazole, cefoxitin, clarithromycin, linezolid, and tobramycin (*M. chelonae* only).
- Testing for additional drugs may be useful toaid in deciding the use of alternative agents for treatment.

Baseline Evaluation at Initiation of Therapy for NTM

Laboratory test	Target population
Complete blood count	All patients
Comprehensive metabolic panel	All patients
Electrocardiogram to assess QT interval	Patients receiving azithromycin, fluoroquinolone, clofazimine
Audiogram	Patients receiving a macrolide or aminoglycoside
Visual acuity and color discrimination	Patients receiving ethambutol, linizolid

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Mycobacterium tuberculosis and nontuberculosis mycobacteria co-infection: Two cases from the sub-Himalayan region of North India in a year

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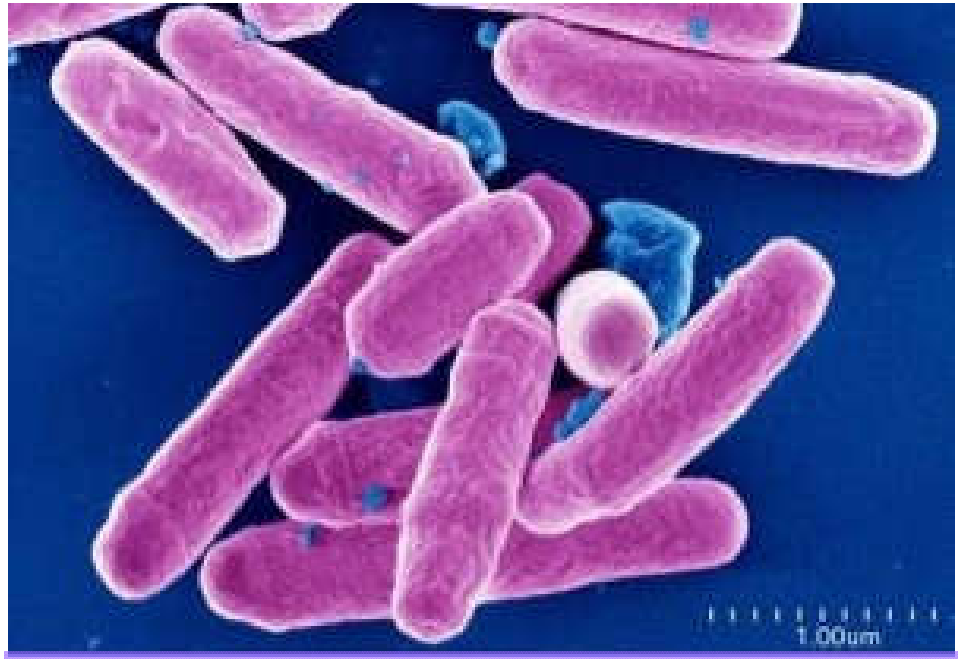
Lung India 2017;34:494-6

As NTM are ubiquitous organisms their mere isolation from pulmonary samples is insufficient evidence for the presence of NTM lung disease. Therefore, diagnosis should rely on clinical, radiographic, and microbiological criteria. Pulmonary TB patients who are non-responders to standard ATT regimen should be evaluated for NTM co-infection and standard therapy for NTM initiated at the earliest.

Tests Done in SRL

TEST	METHOD	CODE
ACID FAST BACILLI CULTURE: MGIT	FLUORESCENT STAIN/ ZIEHL NEELSEN STAIN & MGIT 960+LJ CULTURE	1464/ 1464EP, 1465, 1464S, 1464U
AFB DRUG SENSITIVITY: MIC TEST FOR RAPID GROWING NON-TUBERCULOUS MYCOBACTERIA (NTM)	BROTH MICRODILUTION	1464RGM
AFB SSPECIATION	PCR SEQUENCING	2402



Thank You