

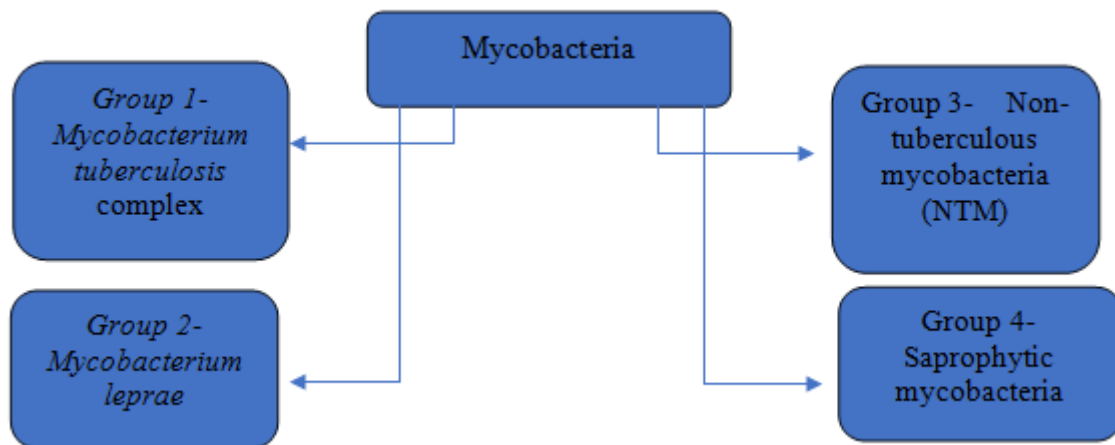
# NON-TUBERCULOUS MYCOBACTERIA

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## I. INTRODUCTION

Mycobacteria is classified into 4 groups based on the species causing human disease, growth rate, capability of causing tuberculosis (TB), and non-pathogenic mycobacteria.



*Figure 1: Classification of Mycobacteria*

**Group 1:** It includes *Mycobacterium tuberculosis* (MTB) complex which includes organisms causing tuberculosis.

**Group 2:** It includes *Mycobacterium leprae*, the organism responsible for causing leprosy

**Group 3:** It includes Nontuberculous mycobacteria (NTM) or atypical mycobacteria, which are ubiquitous, diverse group of mycobacteria isolated from animal, soil, and water originating from natural environments.

**Group 4:** It includes saprophytic mycobacteria which are isolated from environmental sources and do not cause any disease in humans.

Non-tuberculous mycobacteria consist of about 170 species of mycobacteria. They are also called as atypical mycobacteria, which are mycobacteria other than tubercule bacilli/leprosy. NTM have been classified into 4 groups by Runyon based on pigment production and growth rate. NTMs are characterized by the presence of similar morphology to MTB, i.e; presence of outer lipid-rich coating that enables resistance to dyes, antibiotics, disinfectants etc. <sup>[1, 2]</sup>

## II. EPIDEMIOLOGY OF NTM INFECTIONS

NTM are present widely in the environment from soil, water, infections to humans is generally acquired by exposure to environmental contaminants. Most of the pulmonary NTM infections resemble to that of TB and hence it is difficult to differentiate NTM from MTB without proper diagnosis. Recently NTMs are gaining more importance as there is an increase in comorbid conditions and they cause infections in HIV/AIDS, diabetes, renal failure, transplant recipients and immunosuppressive therapy, lung disorders individuals. NTMs may cause both symptomatic and asymptomatic infections and can cause infections in pulmonary and extrapulmonary regions ranging from infection of the skin and soft tissue which occurs following any breach through the skin, device associated infections like infections caused due to catheters, bloodstream infections caused due to central line. But the most common site of infection is lungs and mostly causes pulmonary disease. Pulmonary disease due to NTM usually occurs in cases of COPD, cystic fibrosis, prior infection with TB, bronchiectasis, are some of the predisposing conditions to NTM infections. Followed by MAC are *M. kansasii*, and *M. abscessus* species which come under organisms causing NTM infections worldwide. Incidence rates of NTM infections vary from 1-1.8 cases per 100,000 people. Elderly women are (59%) more prone to pulmonary NTM infections than younger men, and MAC complex is the commonest organism to cause infection.<sup>[3,4]</sup>

Diagnosis of NTM infections is challenging as NTMs are present in the environment and it becomes obligatory to distinguish and isolate NTM species from a suspected individual as a causative agent. Often, NTMs are isolated as colonizer/commensal in suspected cases without causing any pulmonary symptoms. This brings the need to classify pulmonary NTM infections from contamination with environmental NTM species. The American Thoracic society (ATS) and Infectious diseases Society of America (IDSA) has proposed few sets of guidelines to identify and classify pulmonary NTM infections. They include-

- Clinical findings  
Presence of pulmonary symptoms, like presence of nodular or cavitary opacities on chest radiograph, or an HRCT scan showing multifocal bronchiectasis with multiple small nodules.
- Microbiological findings  
Positive culture results must be isolated either from 2 sputum samples, or from a sterile site showing pathological modifications like granulomatous inflammation. Sterile samples include bronchial wash/lavage/transbronchial/lung biopsy.
- Strain identification to confirm NTM disease.
- Patients suspected of having pulmonary NTM disease, but do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded.<sup>[5]</sup>

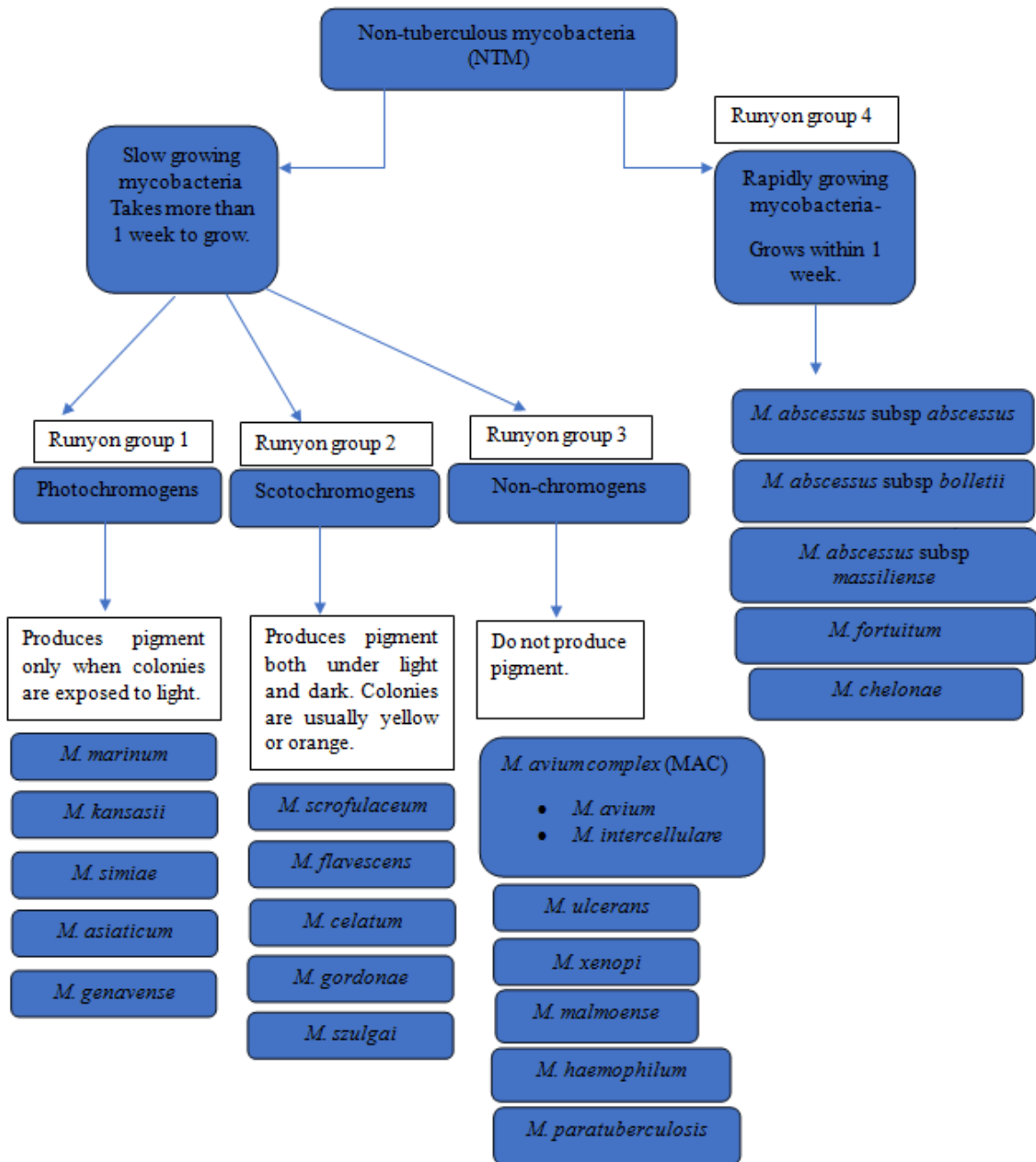


Figure 2: LFlow diagram illustrating classification of Non-tuberculous mycobacteria

Table 1: Different Species of NTMs

Organisms	Clinical Correlation
<b>1)Slow growers</b>	
<b>Photochromogens (Runyon group 1)</b>	
<i>M. kansasii</i>	Infection caused by <i>M. kansasii</i> resembles tuberculosis infection. Chronic pulmonary disease is caused which usually occurs in the upper lobes. Extrapulmonary manifestations are less common,

	although cases of scrofula-like lymphadenitis, sporotrichosis-like cutaneous infections, osteomyelitis, soft tissue infections, and tenosynovitis have been reported. Disseminated infection is more common in the case of AIDS.
<i>M. marinum</i>	Infections usually occurs involving skin, when exposed/traumatized skin comes in contact with freshwater, swimming pools, aquariums, water cooling towers. Lesions are present as tender, red or blue-red subcutaneous nodules usually involving elbow, knee, toe or finger. usually called as “swimming pool granuloma”.
<i>M. simiae</i>	Infection caused by <i>M. simiae</i> are few. Organisms are rarely recovered from blood, jejunal fluid, and duodenal and rectal biopsies. Disseminated disease, with renal involvement following pulmonary infection has also been reported. It has been reported from cases of AIDS.
<i>M. asiaticum</i>	<i>M. asiaticum</i> rarely causes any infection in humans <i>M. asiaticum</i> has been isolated from fluid aspirated from an olecranon bursa in a patient with a post-surgery infection. From a report published by Australia, 2 of the 5 patients had progressive cavitory pulmonary disease; three had no evidence of progressive pulmonary disease.
<i>M. genavense</i>	It is an uncommonly recovered organism. Most of the infections are caused in cases of AIDS. In individuals with AIDS, it produces lesions characterized by masses of foamy histocytes and the granulomas are usually ill-defined. Disseminated disease is usually seen with the involvement of spleen, liver, lymph nodes.
<b>Scotochromogens (Runyon group 2)</b>	
<i>M. scrofulaceum</i>	It causes scrofula (cervical lymphadenitis) in children between 18 months and 7 years of age, a time when oral mucous membrane barrier breaks because of teething and colonization occurs during teething.
<i>Mycobacterium celatum</i>	<i>M. celatum</i> closely resembles <i>M. xenopi</i> phenotypically. It has been recovered from respiratory specimens, and less commonly from the blood, stool, and cerebrospinal fluid.
<i>M. gordonae</i>	<i>M. gordonae</i> rarely causes human infections. It is found particularly in aqueous environments, hence the alternative name <i>M. aquae</i> , or the “tap water bacillus” is designated. It is most often a contaminant. It has been reported from cases of ventriculoarterial shunts, hepato-peritoneal disease, endocarditis in a prosthetic aortic valve, cutaneous lesions of the hand, and possibly patients with pulmonary involvement. Disseminated disease also occurs.

<i>M. szulgai</i>	Lung infections with cavitation resemble <i>M. tuberculosis</i> . Symptoms like fever, cough, haemoptysis, and weight loss will be seen. Extrapulmonary infections with <i>M. szulgai</i> include olecranon bursitis, tenosynovitis, carpal tunnel syndrome, osteomyelitis, and localized cutaneous disease.
<b>Non- chromogens (Runyon group 3)</b>	
<i>M. avium complex</i>	Predisposing conditions to involvement of pulmonary infections with MAC complex includes COPD, bronchiectasis, previous infection with TB, pneumonia etc. Pulmonary manifestations of MAC disease are similar to <i>M. tuberculosis</i> pulmonary symptoms. MAC organisms also cause a scrofula-like cervical lymphadenitis in children. Members of the MAC can be recovered from many environmental sources, including water estuaries, pools, soil, pools, house dust, plants. Human MAC infections may occur from ingestion of contaminated water and food or by inhalation of organisms contained within aqueous aerosols. Although poultry, swine, and other species of birds and animals become infected and excrete organisms in the faeces that can remain viable for long periods in soil, animal-to-human transmission is rare, and no human-to-human transmission has been documented. MAC has a worldwide distribution; however, endemic areas have been found in temperate geographic areas, including the United States, Canada, Great Britain, Europe, the Netherlands, and Japan. Members of MAC are the NTM most frequently associated with human disease.
<i>M. ulcerans</i>	Infection with <i>M. ulcerans</i> , termed Buruli ulcer, typically is present as a “boil” or lump under the skin on the lower extremities, which usually develops at the site of previous trauma. Later this develops into a shallow, nonhealing ulcer, with a necrotic base after sometime. They are generally painless, unless they become secondarily infected with bacteria. Some lesions may be quite severely painful.
<i>M. xenopi</i>	<i>M. xenopi</i> has been isolated from water taps, water storage tanks, hot water generators of hospitals, and these water equipments are potential sources for nosocomial infections. <i>M. xenopi</i> majorly causes pulmonary infections, with a very close resemblance with that of <i>M. tuberculosis</i> , <i>M. kansasii</i> , or MAC infections. Radiographic findings include multinodular densities, cavities, and fibrosis. Infections usually occur in patients with pre-existent lung disorders or predisposing conditions, such as alcoholism, malignancy, and diabetes mellitus.
<i>M. malmoense</i>	It causes pulmonary disease and young children are mostly affected with cervical lymphadenitis. It also affects adults with underlying conditions such as COPD and rarely extrapulmonary diseases and

	disseminated infections are documented.
<i>M. haemophilum</i>	It required 0.4% hemoglobin/ 60 μM hemin for growth. Most of the infections involve skin, subcutaneous tissue, involvement of bone, joints, lymphatics and most commonly infects individuals with AIDS. Common clinical presentations include painful subcutaneous nodules, inflammation, swelling with nodules progressing to ulcers and ulcers to abscesses and draining fistulas.
<i>M. paratuberculosis</i>	This organism has been linked with pathogenicity of Crohn's disease. It has been recovered from the intestinal mucosa of infected animals, initially known as Johne's bacillus but later identified as <i>M. paratuberculosis</i> .
<b>2) Rapid growers Runyon group 4</b>	
<i>M. fortuitum</i> , <i>M. chelonae</i> <i>M. abscessus</i> group	This group organisms are clinically important organisms. Infections of the skin and subcutaneous tissue have been majorly documented (which are the most common) followed by, bone, lung, central nervous system, cardiac infections and disseminated disease have been documented. Risk factors for acquisition of these infections are organ transplantation, rheumatoid arthritis, presence of other autoimmune disorders, and other factors consist of skin trauma including medical procedures which require surgical treatment. RGM may cause pneumonia, with pulmonary infections caused by <i>M. abscessus</i> being particularly serious. RGM are ubiquitous in many water sources and may colonize the respiratory tract of patients who have compromised local defense mechanisms, who are debilitated or immunocompromised, or who have long-standing chronic obstructive pulmonary disease. <i>M. chelonae</i> infections include otitis media due to transfer of the organism between patients from contaminated instruments, aortitis following aortic valve replacement, sternal wound infection, endocarditis, cardiac bypass-related infections, hepatitis, synovitis, retroperitoneal abscess.
Rapidly growing mycobacteria that rarely cause human infections are <i>M. mucogenicum</i> group ( <i>M. mucogenicum</i> , <i>M. auagnense</i> , and <i>M. phocaicum</i> ), <i>M. mageritense</i> , <i>M. wolinskyi</i> . <sup>[3,6,7]</sup>	

### III. LABORATORY PROCEDURE FOR COLLECTION OF SPECIMENS

- 1. Specimens for Mycobacterial Infections are Collected from the Site of Infection:**  
Mycobacteria can infect any part of the body other than lungs and causes extrapulmonary manifestation.

Based on the site of infection, body fluids, tissues, abscesses are collected in an aseptic manner through use of needle aspirations which required surgical expertise and these

procedures are employed to minimize the rates of contamination and effective recovery of the involved organism. Based on the site involved, CSF, synovial fluid in case of bone/joint/ skeletal infections, pericardial fluid in case of pericarditis, tissue samples are collected. In case of suspected pulmonary NTM infections, sputum, Bronchoalveolar lavage fluid (BAL), Endotracheal aspirate samples (ET aspirate), Induced sputum (IS) are usually collected. In children, Gastric lavage (GL) is collected as they are unable to expectorate sputum. Out of all the respiratory samples, sputum is the most preferred sample. A good and early detection of NTM infection involves the correct type of the specimen to be collected in an appropriate amount contamination must be avoided by taking proper care and guidance.

For the collection of sputum sample, it is generally recommended to collect 3 sputum samples, where, 2 sputum samples can be collected within the same day with a time gap of 1-2 hours. The other sputum sample is collected in the early morning and is considered as the best, as overnight incubation will allow the isolation of the bacilli more efficiently. Patient has to be instructed as of how to expectorate sputum.

**2. Instructions for Expecting a Sputum Sample:** The patient is advised to rinse the mouth with sterile water to avoid contamination. Sputum must be generated from a deep cough within the lungs, and saliva is not an appropriate sample as it contains normal flora and can cause contamination. Patient must be instructed to cough inside the screw-capped container such that no aerosols are produced. 5-10ml of sputum must be collected in a leak-proof, sterile container and must be sent to the lab without any delay. Screw-capped containers must be capped tightly to avoid spillage of sputum outside the container. If delay is suspected, samples should be refrigerated.<sup>[8]</sup> Labelling with the patient's demographic information, date and time of collection must be carried out, along with that, patient's form must be sent to the laboratory for the required tests to be performed.<sup>[9]</sup> Sputum samples are collected from the patient only if they are capable of producing it, if patients are not able to produce sputum, other samples like Bronchoalveolar lavage fluid, ET aspirates can be collected which is an invasive procedure and is to be done in proper assistance. For a proper sputum sample to be collected, patients must be given the following instructions: -

- Assistance on expectoration of sputum must be given prior to the collection.
- Patient must be advised to rinse water with sterile water and not to use any toothpaste as it can cross-contaminate.
- Proper opening and handling of the sterile screw-capped bottle.
- Patient must be advised to expectorate sputum sample in a well-ventilated area, far away from the hospital/lab area.
- Patient, must be advised to take deep breath before expectorating sputum and must be told that saliva is unacceptable, and carefully spit the sputum into the container.
- Patient must be told that the sputum must not leak from the container and advised to close the container tightly. Clean the outside of the container with 5% phenol.
- Wash hands after collection of the sample.

**3. Digestion, Decontamination and Concentration:** Digestion, decontamination and concentration procedures are usually done to minimize contamination. The most widely used digestion-decontamination method is by using N-acetyl-l-cysteine-sodium

hydroxide (NALC-NaOH) which acts as a compound that makes mucous less thick by disrupting chemical bonds in mucous. Therefore, it makes sputum less thick. The digestion and decontamination process utilizing the compound NALC combined with 4% NaOH and 2.9% trisodium citrate solution helps to kill all the other organisms except for mycobacteria. This method is often used in conjunction with a 5% oxalic acid procedure (“double processing”) for specimens from patients with CF or bronchiectasis whose sputa are known to be contaminated with aerobic gram-negative rods. Following this procedure, microscopy is done to examine the slides for the presence of mycobacteria. <sup>[10,11]</sup>

#### IV. LABORATORY DETECTION METHODS

1. **Smear Microscopy:** The most sensitive method for detection of AFB (MTB and NTM) is the fluorescent staining technique although, Ziehl-Neelsen staining technique and Kinyoun’s staining are acceptable, but they are less sensitive.
2. **Auramine O Phenol Staining Method:** It is a fluorescent staining technique, which uses Auramine phenol solution as a primary stain. Phenol helps in the penetration of the primary stain. When Auramine is added, it forms a complex with mycolic acid, and resists decolourisation by acid-alcohol. Potassium permanganate, a counterstain stains the debris and the background. Acid-fast organisms appear slender, bright golden-yellow/yellowish-green rods against a dark background. <sup>[12]</sup> Smear grading of the sample is done according to the number of bacilli present.
3. **Ziehl – Neelsen Staining-** Property of acid-fastness is because of the presence of a waxy component, mycolic acid which is a component of the cell wall. When the primary stain (carbol fuchsin) is added, it binds to the cell wall mycolic acid and resists decolorization even on adding acid, and as a result, primary stain is not released, and retains the pink colour of carbol fuchsin. On counter staining, it provides a blue background. Acid-fast organisms are seen as long and slender, beaded, less uniformly stained, red coloured.

**Table 2:** Grading of the Smears- Fluorescent Staining Technique and Ziehl-Neelsen Staining Technique.

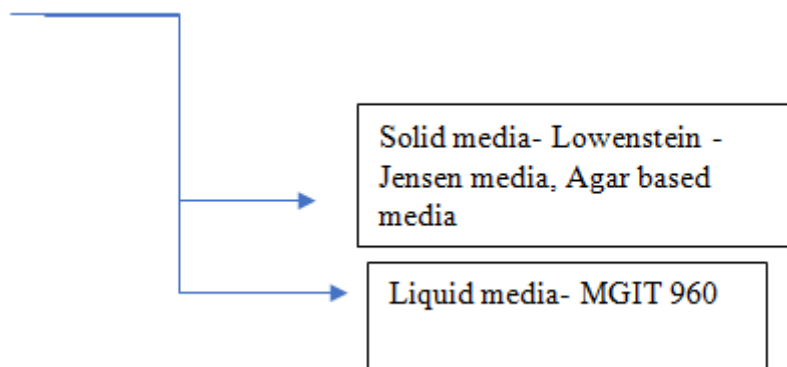
Reference from- Manual for Sputum Smear Fluorescence Microscopy- RNTCP, Central TB division <sup>[10]</sup>

<b>RNTCP ZN Staining Grading (using 100x Oil Immersion Objective and 10x Eye Piece)</b>	<b>Auramine O fluorescent Staining Grading: (using 20 or 25x Objective and 10x Eye Piece)</b>	<b>Reporting/Grading</b>
>10 AFB/ HPF- after examination of 20 fields.	>100 AFB/field after examination of 20 fields	Positive, 3+
1-10 AFB/HPF- after examination of 50 field.	11-100 AFB/field after examination of 50 fields	Positive, 2+
10-99 AFB/100 HPF	1-10 AFB/ field after	Positive, 1+



	examination of 100 fields	
1-9 AFB/ 100 HPF	1-3 AFB/100 fields	Positive, scanty/ doubtful repeat.
Zero AFB/100 HPF	No AFB per 100 fields	Negative

## V. CULTURE OF MYCOBACTERIA



- 1. Solid Media- LJ Media:** Culture through Lowenstein-Jensen media is considered gold standard and sensitive, it is more time consuming when compared to microscopy as it requires 4-8 weeks to produce colonies. It is an egg-based media which consists coagulated hen's eggs, asparagine, malachite green, glycerol, salt solution.<sup>[10]</sup>
- 2. Liquid Media- MGIT 960:** It includes a liquid culture medium which is supplemented with factors essential for growth, and mixture of antibiotics to inhibit the contamination by normal flora. Each MGIT tube contains 100 µl fluorescent indicator which contains Tris 4,4 diphenyl-1,10 phenanthroline ruthenium dichloride pentahydrate in a rubber base which is made of silicon. Tubes are flushed with 10% CO<sub>2</sub> and capped with polypropylene caps. OADC- Oleic acid, bovine albumin, dextrose, catalase, for enriching the growth of tubercule bacilli and PANTA- Polymyxin b, Amphotericin b, Nalidixic acid, Trimethoprim, Azlocillin. Each tube contains 7ml of middle brook 7H9 solution. The base of the tube is comprised of a fluorescent compound, and the fluorescent component is sensitive to oxygen. When microorganisms respire, oxygen is utilized and as oxygen is reduced, the fluorescence can be detected. Inoculated MGIT tubes are incubated at 37 °C, for 4 weeks and read manually under ultraviolet light or incubated into MGIT 960 instrument and monitored for fluorescence.<sup>[13]</sup>

### Identification of NTM

NTMs can be categorized based on the growth rate, pigmentation, selection of proper testing procedures. As mentioned earlier, NTM species taking less than 7 days to grow are classified as rapidly growing mycobacteria. Isolates requiring more than 1 week to produce colonies on the media are classified as slow-growing mycobacteria. Based on production of pigment, they are again classified into 3 groups.

## VI. GENOTYPIC METHODS

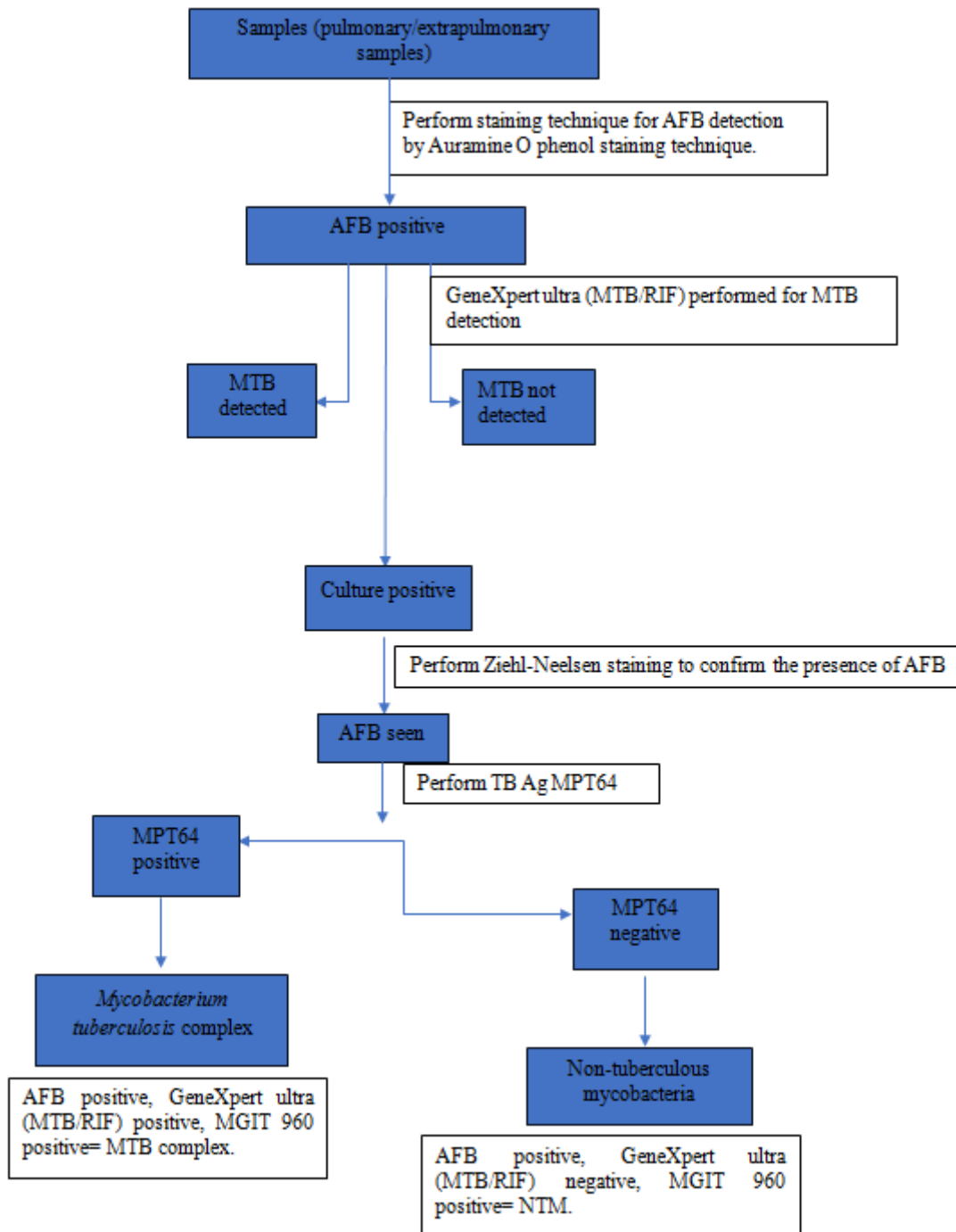
- 1. Nucleic-Acid Probes:** It is used to identify the target organism from positive cultures. Nucleic acid probes are used for identifying different MTB complex and also NTM species like *M. kansasii*, *M. gordonae*. Different probes are available even for *M. avium*, *M. intracellulare*. This innovation uses acridine ester-labelled single-stranded DNA probes which hybridize with the ribosomal RNA (rRNA). With the help of a lysing agent, action of heat and sonification, the rRNA is released and it is used as a target for the identification. The release rRNA combines with the DNA probe and hybridization takes place and the fluorescence is generated which is captured by the instrument. This test can be executed from solid/liquid culture isolates and the result is acquired within 2 hours. Probes are not available to all the NTM species which is a limitation, but the test has a very high rate of sensitivity and specificity.
- 2. DNA Sequence Analysis:** For mycobacterial identification purposes, DNA sequence analysis focusses on two hypervariable sequences known as region A and region B. Region A sequence is used to identify NTM species and region B is used for the identification of undescribed species or those species which cannot be differentiated by sequence of region A alone. They include *M. kansasii*, *M. ulcerans*, *M. marinum*.<sup>[14]</sup>
- 3. PCR Assays-Genexpert Ultra (MTB/RIF):** GeneXpert ultra (MTB/RIF) is a nucleic acid amplification test, used to amplify the MTB DNA, and to determine MDR-TB (Rifampicin resistance detection) in pulmonary and extrapulmonary samples. Real-time PCR is the principle of GeneXpert ultra (MTB/RIF), which helps to detect, and amplify the target sequences by melt curve analysis. Each test sample includes a SPC and PCC (Sample processing control) and (Probe check control). The formerly known categories were “high,” “medium,” “low,” and “very low,” now a recently developed class called “trace” is included by GeneXpert ultra (MTB/RIF) system and can detect trace amounts of the MTB DNA which can tell the severity of the infection. SPC (Sample processing control) is embedded in the cartridges and when the sample is inoculated into the equipment, SPC combines with the sample, and helps in lysing of the cells if present and it is captured by the filter where both sample and the SPC and ultrasonically lysed cells, releases DNA, which is detected along with rifampicin resistance. This test can detect the presence of MTB DNA and not NTM species.<sup>[15]</sup>

If the test sample shows positive smear and negative GeneXpert ultra (MTB/RIF) and positive culture, a rapid test must be performed.

- 4. Line Probe Assay:** It is based on PCR technique, where the hybridization technique occurs on a nitrocellulose membrane and multiple probes are designed according to the target sequences and are immobilized on the nitrocellulose paper. Target sequences are amplified by the primers and multiple probes are immobilized on the strip, and when the amplified product is applied to the strip, lines are produced at the site of hybridization. This pattern is later compared and interpreted with the help of a key.<sup>[16]</sup>

This technique is helpful in the rapid identification of mycobacteria including MTBC, MAC, and the other mycobacterial species: *M. avium*, *M. intracellulare*, *M. kansasii*, *M. chelonae* group, *M. gordonae*, *M. xenopi*, and *M. scrofulaceum*, *M. abscessus*.

5. **MPT64 Test:** *M. tuberculosis* produces more than 33 different proteins, out of it is MPT64 is one of them. This test is a rapid ICT and is performed for all the positive MGIT 960 tubes to differentiate *Mycobacterium tuberculosis* from Non-tuberculous mycobacteria. It consists of a sample pad, a nitrocellulose membrane, and an absorbent pad. Mouse monoclonal anti-MPT64 are immobilized on the nitrocellulose membrane as test line. This test principle captures and detects target based on sandwich type assay. It requires addition of 100µl of sample (from MGIT 960 tube) into a sterile pipette into the sample well after a dense mixing from the colonies. Test results will be ready after 15 minutes. If both control band and test band is visible, the test is considered positive. If only control band is visible, test is considered negative. If the control band is not visible, the test is considered invalid. If the test is negative, but the culture tubes are positive for growth, MPT64 rapid immunochromatographic test must be performed since NTMs do not produce this protein.



**Figure 3:** Flow diagram for identification and differentiation of MTB from NTM.<sup>[17]</sup>

### Antimicrobial Susceptibility Testing for NTM

It is important to determine the drug susceptibility patterns for NTM to lock with a specific anti-microbial treatment. When compared to MTB, drug susceptibility for NTM is difficult because of inconsistent results observed between in vitro testing and in vivo clinical outcomes.

1. **For Isolates of MAC (*M. avium*, *M. intracellulare*):** CLSI has recommended broth-based method (microdilution/macrodilution). For the susceptibility testing, Clarithromycin is the drug is recommended in case of new and previously untreated MAC isolates. For untreated MAC isolates have a MIC of 4 µg/ml or less to clarithromycin and are considered susceptible. Relapse strains after treatment have a MIC of 32 g/ml or greater to clarithromycin and no longer respond to treatment with macrolides.
2. ***M. Kansasii*:** CLSI has recommended to use rifampin against isolates of *M. kansasii*. If the isolate is rifampin resistant, use of secondary agents, like amikacin, ciprofloxacin, clarithromycin, ethambutol, rifabutin, streptomycin, sulfonamides, and isoniazid, should be tested for susceptibility patterns.
3. ***M. Marinum*:** Routine susceptibility testing of this species is not recommended as there is no documentation as there is no antimycobacterial agents and there is no variability in susceptibility patterns to antimycobacterial agents. However, *M. marinum* is susceptible to clarithromycin, sulfonamides, tetracyclines, rifampin, ethambutol.
4. ***M.simiae*, *M. Malmoense*, *M. Xenopi*:** Since the cases are very less reported and very few isolates are studied, no specific susceptibility is recommended.
5. **Rapid Growers:** CLSI has recommended the microbroth dilution method for susceptibility testing. In case of *M. abscessus* *M. chelonae* isolates, imipenem is the preferred carbapenem over meropenem and ertapenem, although imipenem is clinically important. In-vitro susceptibility studies suggest that tobramycin is the most active aminoglycoside for *M. chelonae*, so tobramycin MIC has to be reported for its species. For *M. fortuitum* group with indeterminate susceptibility pattern with clarithromycin, it has to be reported as resistant. But recent studies have shown that, *M. fortuitum* contain an inducible erm gene, which is erythromycin methylase gene, due to which there is macrolide resistance.
6. **Fastidious Species of NTM- *M. Haemophilum*:** These isolates are susceptible to all the first line anti-tubercular drugs except ethambutol. They are also susceptible to clarithromycin, sulfonamides.
7. ***M. Genavense*, *M. Avium Subsp*, *M. Paratuberculosis*, *M. Ulcerans*:** These organisms do not grow in standard susceptibility media, so it is difficult to perform susceptibility pattern.<sup>[5, 14]</sup>

**Table 3: Treatment Strategies for NTM Infections**

(From American Thoracic S. and Infectious Disease Society of A. An Official ATS/ IDSA Statement: Diagnosis, Treatment, and Prevention of Non-Tuberculous Mycobacterial Diseases)

NTM Species	American Thoracic Society Guidelines	British Thoracic Society
<i>M. abscessus</i>	Oral macrolides+ 2 parenteral drugs. Amikacin, Cefotaxime or Imipenem	Daily intravenous injection of Amikacin 15mg/kg, Tigecycline

	for 12 months.	50mg, 1g Imipenem+ oral macrolide 250-500 mg. (all twice)  Continuation phase- Daily  Nebulized amikacin, oral macrolide 250-500 mg+ oral clofazimine 50-100 mg; oral linezolid 600 mg, oral minocycline 100 mg; oral moxifloxacin 400 mg daily; oral cotrimoxazole 960 mg (all twice).
<i>MAC complex</i>	3 times weekly regimen containing Clarithromycin 1000 mg, azithromycin 500 mg, Ethambutol 25 mg/kg, Rifampin 600 mg is recommended. In case of severe cavitory disease, addition of an injectable aminoglycoside like-Amikacin, Streptomycin is required.	3 times weekly regimen of Rifampicin 600mg, Ethambutol 25mg/kg, Azithromycin 500mg or Clarithromycin 1g.  In case of severe MAC cases, Daily regimen of Rifampicin 600 mg, Ethambutol 15mg/kg, Azithromycin 250 mg or Clarithromycin 500 mg twice (12 months). Nebulized Amikacin or Intravenous Amikacin for 3 months.
<i>M. kansasii</i>	Daily regimen containing Rifampin 10mg/kg, Ethambutol 15 g/kg, Isoniazid 5 mg/kg, Pyridoxine 50mg. In case of Rifampin resistance, 3 drug regimen is recommended along with Clarithromycin, Azithromycin, Moxifloxacin, Ethambutol, Sulfamethoxazole/ Streptomycin.	Daily routine of Rifampicin 600 mg, Ethambutol 15 mg/kg, Azithromycin 250 mg or Clarithromycin 500 mg twice to be included as an alternative to Isoniazid combined with Pyridoxine.  For drug resistant <i>M. kansasii</i> - Same as for ATS/IDSA guidelines.
<i>M. malmoense</i>	Isoniazid, Rifampin, Ethambutol with and without quinolones and macrolides.	3 drug-regimen daily including Rifampin 600 mg, Ethambutol 15 mg/kg, Macrolide twice 250-500 mg for less severe case.  For a severe case, injectable aminoglycoside like Amikacin or Streptomycin for 3 months or nebulized amikacin can be administered.

<i>M. xenopi</i>	Isoniazid, Rifabutin, Rifampin, Ethambutol, Clarithromycin with/without streptomycin. Moxifloxacin to be substituted for one of any ATT.	4-drug regimen daily consisting of Rifampin 600 mg, Ethambutol 15mg/kg, and a macrolide 250mg-500 mg twice with either a quinolone (ciprofloxacin or moxifloxacin 400mg) or Isoniazid 300 mg + pyridoxine 10mg. In case of severe NTM disease, injectable or nebulized amikacin is added to the above regimen for up to 3 months.
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## VII. PREVENTION OF HEALTH-CARE ASSOCIATED NTM OUTBREAKS

Patients with indwelling central catheters, especially bone marrow transplant recipients, should avoid contact or contamination of their catheter with tap water. Use of tap-water should be avoided during endoscope washing routines, and alcohol must be used for rinsing the materials for disinfecting the equipment. During surgery, tap water must not be used in the operating rooms during augmentation or surgery. Patients must be strictly told not to rinse mouth with tap water before sputum expectoration.

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