Review Article

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Epidemiology, diagnosis & treatment of non-tuberculous mycobacterial diseases

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Non-tuberculous mycobacteria (NTM) are ubiquitously present in the environment, but NTM diseases occur infrequently. NTM are generally considered to be less virulent than *Mycobacterium tuberculosis*, however, these organisms can cause diseases in both immunocompromised and immunocompetent hosts. As compared to tuberculosis, person-to-person transmission does not occur except with *M. abscessus* NTM species among cystic fibrosis patients. Lung is the most commonly involved organ, and the NTM-pulmonary disease (NTM-PD) occurs frequently in patients with pre-existing lung disease. NTM may also present as localized disease involving extrapulmonary sites such as lymph nodes, skin and soft tissues and rarely bones. Disseminated NTM disease is rare and occurs in individuals with congenital or acquired immune defects such as HIV/AIDS. Rapid molecular tests are now available for confirmation of NTM diagnosis at species and subspecies level. Drug susceptibility testing (DST) is not routinely done except in non-responsive disease due to slowly growing mycobacteria (*M. avium* complex, *M. kansasii*) or infection due to rapidly growing mycobacteria, especially *M. abscessus*. While the decision to treat the patients with NTM-PD is made carefully, the treatment is given for 12 months after sputum culture conversion. Additional measures include pulmonary rehabilitation and correction of malnutrition. Treatment response in NTM-PD is variable and depends on isolated NTM species and severity of the underlying PD. Surgery is reserved for patients with localized disease with good pulmonary functions. Future research should focus on the development and validation of non-culture-based rapid diagnostic tests for early diagnosis and discovery of newer drugs with greater efficacy and lesser toxicity than the available ones.

Key words Diagnosis - non-tuberculous mycobacteria pulmonary disease - NTM - NTM extrapulmonary disease - treatment

Introduction

Non-tuberculous mycobacteria (NTM) are known by several names including environmental mycobacteria, atypical mycobacteria or anonymous mycobacteria, mycobacteria other than *Mycobacterium tuberculosis* (*Mtb*) (MOTT) and its close relatives, *M. africanum*, *M. bovis*, *M. canetti, M. caprae, M. pinnipedii* and *M. leprae*[1]. These organisms are ubiquitous in the environment and have been isolated from air, soil, dust, plants, natural and drinking water sources including biofilms, wild animals, milk and food products[2,3]. NTM are characterized by a thin peptidoglycan layer

surrounded by a thick outer lipid-rich coating that enables NTM attachment to rough surfaces and by offering resistance to antibiotics and disinfectants, helping NTM survival in low oxygen and carbon concentrations and in other adverse conditions[4]. Based on their growth characteristics from the subculture, NTM are divided into rapidly growing mycobacteria (RGM; <7 days) and slowly growing mycobacteria (SGM; ≥7 days)[5] (Table I). At present, there is no evidence for the latency of NTM[6]. Taxonomy of the genus *Mycobacterium* includes about 200 species and 13 subspecies[7-9].

In high tuberculosis (TB)-burden countries, diagnosis of NTM is rarely made because of lack of awareness among healthcare providers about the NTM diseases and poor access to adequate laboratory resources including mycobacterial culture and molecular methods for identification or speciation[10]. In these resource-limited settings, there is a heavy reliance on smear microscopy for the diagnosis of TB, and the diagnosis of NTM is frequently missed and these patients are empirically treated as drug-sensitive and -resistant TB[11].

Table I. Common non-tuberculous mycobacteria (NTM) species causing human diseases

Slowly growing NTM (showing growth in ≥7 days on subculture)
1. Photochromogens (produce pigment on exposure to light)
Mycobacterium kansasii
M. marinum
2. Scotochromogens (produce pigment when grown in dark)
M. scrofulaceum
3. Non-chromogens (growth not pigmented)
M. avium complex (MAC)
M. avium
M. intracellulare
M. chimaera
M. ulcerans
M. xenopi
M. simiae
M. malmoense
M. szulgai
M. haemophilum
Rapidly growing NTM (showing growth in <7 days on subculture)
M. abscessus
M. abscessus subspecies *abscessus*
M. abscessus subspecies *bolletii*
M. abscessus subspecies *massiliense*
M. fortuitum
M. chelonae

Source: Ref. 5

Epidemiology

NTM disease burden

Table II describes the distribution of various NTM species in the environment[2,3]. Table III details the major differences between NTM and *Mtb*[1-6,9,12-20]. Although recent reports regarding the transmission of *M. abscessus* and *M. massiliense* have not proven person-to-person transmission, but these are highly suggestive of indirect transmission among cystic fibrosis (CF) patients[21]. Systematic reporting of NTM diagnosis is not done because the disease is not notifiable to public health authorities in several countries[10]. NTM lung infection rates, defined as individuals with NTM-positive cultures and those with defined NTM pulmonary disease (NTM-PD), increase with age[22] and differ considerably among various countries[23-25]. Many studies have suggested an increase in the prevalence rates of NTM over the last four decades[22,25-36]. The data from the USA suggest that the current prevalence of NTM-positive culture ranges between 1.4 and 6.6/100,000 individuals[26], whereas UK data suggest that NTM-positive culture incidence has increased from 4/100,000 to 6.1/100,000 individuals between 2007 and 2012[35] . A study from Canada has reported a significant increase in the prevalence of NTM-PD from 29.3 cases/100,000 in 1998-2002 to 41.3/100,000 individuals tested in 2006-2010[36]. Several factors that have contributed to this increase in the incidence and prevalence are listed in Box I[37,38]. Published reports on rate of NTM isolation from several countries are summarized in Table IV[22,29,30,39-63].

Details of 13 Indian studies published between 1985 and 2019 are summarized in Table V[59-71]. Most of these studies have reported NTM isolation rates from laboratories without describing clinical features and treatment details. Two studies were done exclusively on extrapulmonary specimens and 11 on both pulmonary and extrapulmonary specimens. NTM isolation prevalence varied between 0.38 and 23.7 per cent. Six of these 13 studies reported NTM prevalence ≤1 per cent among TB suspects. Almost all except one study have not provided treatment outcomes. Most of the studies (11/13) were hospital based and had selection bias. A large community-based study from south India conducted at four sites in the pre-HIV era has reported NTM isolation prevalence between 4.5 and 8.6 per cent in the sputum specimens. This variable NTM prevalence can be attributed to the following factors: (*i*) differences

Table II. Environmental niches of non-tuberculous mycobacteria (NTM)

Types of sources	Sources	Commonly isolated NTM
Natural water sources	Streams, rivers, lakes, ponds and seawater	MAC, *Mycobacterium fortuitum, M. chelonae, M. kansasii, M. gordonae, M. xenopi, M. marinum*
Man-made water sources	Drinking water supply pipelines	MAC, *M. kansasii, M. gordonae, M. xenopi, M. abscessus, M. fortuitum, M. chelonae, M. scrofulaceum, M. szulgai*
	Cold and hot water tanks	
	Hot tubs, indoor and outdoor pools	
	Household plumbing, showerheads and faucets	
	Hospital plumbing and water supply	
	Ice machines and commercial ice	
	Bottled drinking water	
Aerosols	Showers, hot-tubs, humidifiers, indoor swimming pools, heater-cooler units in hospitals	MAC, *M. kansasii, M. gordonae, M. abscessus*
Other sources*	Natural soil dust, potting soil, peat moss and domestic dust	MAC, *M. fortuitum, M. chelonae, M. kansasii*

*Contaminated tattoo inks: *M. haemophilum* skin disease; contaminated metal working fluids: *M. immunogenum* skin disease; MAC, *Mycobacterium avium* complex. *Source*: Refs 2, 3

Table III. Differences between non-tuberculous mycobacteria (NTM) and *Mycobacterium tuberculosis* (*Mtb*)

Characteristics	NTM	*Mtb*
Nomenclature	NTM have several names: MOTT, atypical mycobacteria, anonymous mycobacteria and environmental mycobacteria. The preferred name is NTM.	*Mtb* is an important member of MTBC responsible for human TB. Other members include *M. africanum, M. bovis, M. canettii, M. caprae* and *M. pinnipedii*.
NTM species distribution	Nearly 200 species are described using DNA sequencing (a new species is defined as >1% difference in nucleotides); NTM species have regional variation due to climatic and geographical factors.	*Mtb* strains [Beijing (most pathogenic), Cameroon, CAS, EAI, Haarlem, LAM, Manu (Indian), and S] have geographical variation.
Biochemical tests	No single biochemical test is available for the diagnosis of NTM species. Some of the NTM species show positive results with niacin accumulation test (*M. simiae, M. chelonae*), nitrate reduction test (*M. ulcerans, M. szulgai, M. fortuitum, M. smegmatis, M. kansasii*), catalase test (*M. fortuitum, M. chelonae, M. abscessus, M. ulcerans, M. szulgai, M. kansasii*), citrate utilization test (*M. chelonae, M. smegmatis*), urea hydrolysis test (*M. kansasii, M. marinum, M. simiae, M. szulgai, M. scrofulaceum*), McConkey agar (without crystal violet) (*M. fortuitum, M. abscessus*) test and tellurite reduction (*M. avium, M. intracellulare, M. simiae, M. fortuitum, M. abscessus*).	*Mtb* is niacin positive, reduces nitrate and is negative for heat-stable catalase test.
Microscopic morphology	Absence of characteristics serpentine cords in acid-fast smears.	Characteristic serpentine cording seen as rope-like aggregates in which long axis of the bacilli is parallel to the long axis of the cord in acid-fast smears.
Growth characteristics in cultures	Rapidly growing (<7 days) and slowly growing (≥7 days) mycobacteria, growth rates are slower than other bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*).	*Mtb* are slowly growing mycobacteria and take ~2 wk to grow. Ordinary bacteria may take ~20 min to 12-24 h in the laboratory. *Mtb* colonies are rough, cauliflower-like and light buff in colour.

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Characteristics	NTM	*Mtb*
Differential identification	Difficult to differentiate NTM from *Mtb* only on the basis of positive acid-fast smear. Culture is important in differentiating from *P. aeruginosa*, *Staphylococcus aureus*, *Nocardia, Aspergillus* and *Sporothrix*, *etc*.	Both smear and culture should be done.
Transmission	Person-to-person transmission does not occur except for *M. abscessus* among cystic fibrosis patients.	*Mtb* is highly transmissible through airborne route especially in PTB with cavitary disease and high bacillary loads.
Route of entry	Infection occurs mainly by inhalation, ingestion or direct inoculation. Airborne NTM are a major source of entry for NTM-PD. In advanced HIV/AIDS, gut colonization with subsequent haematogenous dissemination occurs.	Smaller cough droplet nuclei (<1-10 µM) carrying *Mtb* reach terminal bronchioles and alveoli and establish infection.
Pathogenicity potential	Opportunistic organisms	Highly pathogenic and obligate parasites
Virulence	Generally, NTM have low virulence. *M. kansasii* is more virulent among NTM.	Highly virulent
Latent infection	No evidence of latent NTM infection	Systematic data are available regarding LTBI especially in low TB-burden countries. Efforts should be made to differentiate between LTBI and active disease in high TB burden settings.
Case notification	It is not essential to notify laboratory confirmed, newly diagnosed NTM cases. NTM disease notification is practiced only in a few countries.	Systematic TB notification is encouraged and the global TB report is published annually on a regular basis by the World Health Organization.
Pulmonary: extrapulmonary disease proportions	Pulmonary: Extrapulmonary 80-90%: 10-20% in HIV-negative. Disseminated NTM disease occurs in severely immunocompromised individuals such as advanced HIV/AIDS.	Pulmonary 80-85%: extrapulmonary 15-20% in HIV-negative and pulmonary 40-50%: extrapulmonary 50-60% in HIV/AIDS.
Risk factors	NTM-PD usually occurs in individuals with pre-existing lung disease or in those with quantitatively impaired mucociliary function or in individuals who are heterozygous for CFTR mutations. Lady Windermere syndrome occurs in post-menopausal non-smoking females with nodular-bronchiectasis, several skeletal abnormalities, increased adiponectin and decreased leptin and oestrogen levels, abnormalities in fibrillin gene, high prevalence of gastroesophageal reflux disease and increased susceptibility to NTM infections.	TB can involve both healthy and destroyed lungs. Risk factors include: malnutrition, tobacco smoking, chronic alcohol intake, diabetes mellitus, overcrowding, HIV/AIDS, head or neck cancer, leukaemia, or Hodgkin's disease, drugs including corticosteroids, TNF-α inhibitors or receptor blocker.
NTM species predilection for various organs	Pulmonary: MAC, *M. kansasii, M. xenopi, M. malmoense, M. abscessus, M. fortuitum M. simiae* Skin: *M. ulcerans, M. marinum, M. abscessus, M. chelonae, M. fortuitum* Soft tissues: *M. chelonae* and *M. fortuitum* Lymphadenitis: MAC but can occur with other NTM species also. Disseminated NTM disease: Most commonly due to MAC but other species can also produce disseminated disease.	No such predilection for body organs is known in TB.

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Characteristics	NTM	*Mtb*
Radiographic patterns in MAC-pulmonary disease	Three types of radiographic patterns occur in MAC NTM-PD: Cavitary: In elderly smokers with COPD patients. NB: Predominantly in post-menopausal non-smoking females; bilateral bronchiectasis, multiple nodules and tree-in-bud appearance on HRCT, some may also have small cavitary lesions. Hypersensitivity pneumonitis-like NTM pulmonary disease due to MAC and *M. immunogenum*.	PTB Primary complex (usually in children) Progressive pulmonary disease Post-primary PTB: Cavitary, atelectasis, consolidation Miliary PTB Sequelae such as fibrotic and calcified lesions
Clinical relevance of NTM isolates in respiratory specimens	Clinical relevance of isolated NTM species versus activity of the underlying pulmonary disease should be assessed. Colonization in the host and contamination in the laboratory must be ruled out. Causality association of the particular isolated NTM species with the pulmonary disease should be carefully established before starting the treatment.	*Mtb* produces both latent TB infection and active disease. Active TB disease must be ruled out appropriately before starting the treatment.
Drug susceptibility testing (DST)	DST for NTM is controversial because of poor correlation between *in vitro* DST pattern and *in vivo* treatment response and outcomes. According to CLSI (2018) guidelines[16], initial and recurrent MAC and *M. kansasii* be tested for DST. Both phenotypic and genotypic DST are performed. For MAC, perform DST against macrolides (clarithromycin as a class agent) and amikacin; for *M. kansasii*, against rifampicin and clarithromycin. RGM species (and subspecies) show different drug resistance patterns and DST should be selectively tested for various antibiotics (macrolides, amikacin, tobramycin, imipenem, trimethoprim-sulphamethoxazole, doxycycline, minocylcine, tigecyline, cefoxitin linezolid) DST, *erm* (41) gene status should be done in *M. abscessus*. Information about *erm* (41) gene and phenotypic DST for clarithromycin should be done on days 3-5 and 14 in case of *M. abscessus*.	Universal DST should be performed and treatment should be carried out as per sensitivity profile of *Mtb*. DS-TB, H monoresistance, MDR-TB and XDR-TB should be treated with as per National Guidelines, and tolerance of drugs.
Treatment	ATS (2007)[17] and BTS (2017)[1] ATS/ERS/ESCMID/IDSA[18] guidelines on NTM diseases should be followed.	National guidelines should be followed for treatment of drug sensitive and drug-resistant TB.
Treatment outcomes	Treatment outcomes differ among NTM species and subspecies.	Globally, treatment outcomes in case of drug-sensitive TB are good. Treatment of drug-resistant TB is still a challenge and global rate of successful treatment is 56% only. With newer drug regimen(s), treatment success rates are likely to improve in future.
Prevention	Exposure to NTM from the environmental sources especially household water systems, hospital settings and soil should be avoided. In HIV/AIDS patients (CD4 T-cells counts <50/µl), antimicrobial prophylaxis includes administration of azithromycin (1200 mg/weekly) or clarithromycin (500 mg twice daily) or rifabutin (300 mg/day) along with antiretroviral drugs till CD4 cell count is >100 cells/µl for three months.	Exposure to smear positive PTB should be avoided to halt TB transmission. Chemoprophylaxis for latent TB infection (active TB disease must be ruled out in high TB-burden countries), various treatment options include: isoniazid daily for 6 or 9 months, or combination of rifapentine and isoniazid once weekly for 12 wk or combination of rifampicin and isoniazid daily for 3-4 months or rifampicin alone daily for four months.

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Characteristics	NTM	*Mtb*
Vaccines	No vaccine is available at present	BCG vaccine is recommended in high TB burden countries to prevent severe form of TB (miliary and central nervous system TB); newer TB vaccines such as M72/AS01, *M. vaccae*, MVA85A *etc*., are in clinical trials. M72/AS01 was significantly protective against TB disease in a Phase IIb trial in Kenya[20].

Disseminated disease: Involvement of two or more non-contiguous body sites through haematogenous route. Note: Underlying oesophageal disease must be ruled out in NTM-PD due to RGM especially *M. fortuitum*. NTM-PD, NTM-pulmonary disease; MTBC, *Mtb* complex; CAS, Central Asian strain; EAI, East African Indian strain; LAM, Latin American-Mediterranean strain; COPD, chronic obstructive pulmonary disease; SGM, slowly growing mycobacteria; RGM, rapidly growing mycobacteria; CLSI, Clinical and Laboratory Standards Institute; ATS, American Thoracic Society; BTS, British Thoracic Society; ATS/ERS/ESCMID/IDSA, American Thoracic Sciety European Respiratory Society European Society of Clinical Microbiology and Infectious Diseases Infectious Diseases Society of America; *erm*, erythromycin ribosome methylation; MOTT, mycobacteria other than TB; LTBI, latent TB infection; CFTR, cystic fibrosis transmembrane conductance regulator; TNF, tumor necrosis factor; MAC, *Mycobacterium avium* complex; NB, nodular/bronchiectatic; HRCT, high-resolution computed tomography; MDR, multidrug resistant; XDR, extensively drug resistant; PTB, pulmonary TB; BCG, bacille Calmette-Guerin. *Source*: Refs 1-6, 9, 12-20

Table IV. Global prevalence of pulmonary non-tuberculous mycobacteria (NTM) isolation and NTM disease

Zone	Countries	NTM isolation prevalence per 100,000 individuals	NTM disease prevalence per 100,000 individuals	Commonly isolated NTM species
North America	Canada[36]	22.2	9.08	MAC, *Mycobacterium xenopi, M. abscessus, M. fortuitum, M. chelonae, M. gordonae*
	USA			
	Oregon[39]	12.7	8.6	
	California[39]	191	NR	MAC, *M. kansasii, M. abscessus, M. xenopi, M. fortuitum*
	Hawaii[22]	396	NR	
South America	Brazil[40]	1.31	0.25	MAC, *M. kansasii, M. abscessus, M. xenopi, M. fortuitum*
Europe	Ireland[41]	1.9	0.2	MAC, *M. kansasii, M. xenopi, M. malmoense, M. marinum, M. szulgai, M. gordonae, M. abscessus, M. chelonae*
	Scotland[42]	NR	3.1	
	The United Kingdom[43]	2.9	1.7	
	Denmark[34]	2.5	1.1	
	Netherlands[44]	6.3	1.4	
	France[45]	NR	0.7	
	Greece[46]	07	0.7	
	Croatia[47]	5.3	0.75	
Oceania	Australia[48]	5.9	0.56	MAC, *M. kansasii, M. abscessus, M. fortuitum, M. simiae*
	New Zealand[49]	3.7	0.56	
Africa	Kenya[50*]	1.7%	NR	MAC, *M. abscessus, M. malmoense, M. marinum, M. xenopi, M. scrofulaceum, M. simiae, M. gordonae*
	Nigeria[51*]	4.3%	NR	
	Uganda[52*]	4.3%	NR	
	Burkina Faso[53*]	20.6%	NR	
Asia	Japan[29]	33-65	NR	MAC, *M. abscessus, M. fortuitum, M. simiae, M. szulgai, M. chelonae, M. gordonae*
	South Korea[54]	39.6	NR	
	China[55*]	6.3%	NR	
	Taiwan[30]	7.94	NR	
	Singapore[56]	511	NR	
	Iran[57,58*]	0.7 to 8%	NR	
	India[59-63*]	0.2 to 5.9%	0.8%	

*Data presented in % is the isolation of NTM among TB suspected individuals in high TB burden countries
Note: NTM isolation data for India provided from Refs 59-63 and disease prevalence from Ref. 61
NR, not reported; MAC, *Mycobacterium avium* complex

Box I. Factors contributing to increased non-tuberculous mycobacteria burden

1. Genetic evolution in NTM due to mutations leading to increased virulence
2. Environmental and climatic changes due to increased human-manufactured infrastructure
3. Changes in host immunity due to increased life expectancy and immunocompromised population
4. Increased incidence of chronic lung disease
5. Decreasing herd immunity due to declining TB burden especially in high-income countries
6. Widespread availability of CT scanning and laboratory infrastructure for NTM diagnosis
7. Increasing awareness among medical personnel about NTM disease
8. Sharp rise in NTM publications by laboratories and practicing physicians

CT, computed tomography; NTM, non-tuberculous mycobacteria. *Source*: Ref. 38

Table V. Summary of Indian studies on non-tuberculous mycobacteria (NTM)

Study details	Methods of NTM detection and identification and results	Identified NTM species	Limitations
	North zone		
Myneedu *et al*[59], New Delhi Hospital-based prospective study (2009-2011) Total TB suspects=15,581 PTB=12,466 EPTB=3,115 HIV status: Not available	ZN staining Liquid culture (MGIT 960) Biochemical tests Prevalence: 0.38% (60/15581) in TB suspects Other results: Pulmonary NTM: 45% (27/60) Extrapulmonary NTM: 55% (33/60)	21 NTM species were identified, % (n) *Mycobacterium simiae* 11.3 (7) *M. avium* 9.7 (6) *M. gordonae* 8.1 (5) *M. kansasii* 8.1 (5) *M. fortuitum* 8.1 (5) Others: *M. chelonae* 8.1 (5), *M. phlei* 8.1 (5), *M. terrae* 6.4 (4), *M. szulgai* 3.2 (2), *M. vaccae* 3.2 (2), *M. flavescens* 3.2 (2), *M. trivale* 3.2 (2), *M. malmoense*, *M. scrofulaceum*, *M. intracellulare*, *M. xenopi*, *M. ulcerans*, *M. tusciae*, *M. triplex*, *M. septicum*, *M. mucogenicum* each 1.6 (1)	Clinical relevance of isolated NTM is not established. HIV status of patients not provided. Molecular methods such as PCR and gene sequencing not performed for NTM species identification. Treatment details including outcomes not provided.
Jain *et al*[60], New Delhi Hospital-based retrospective study (2011-2012) Total TB suspects=436 PTB=237 EPTB=199 HIV status: All negative	ZN staining Liquid culture (MGIT 960) PNB-LJ culture ICA (SD MPT64TB Ag Kit) Multiplex-PCR Prevalence: 2.98% (13/436) Other results: Pulmonary NTM: 69.2% (9/13) Extrapulmonary NTM: 30.8% (4/13)	*M. kansasii* 30 (4) *M. chelonae* 23.1 (3) *M. xenopi* 15.4 (2) *M. scrofulaceum* 7.7 (1) *M. avium* 7.7 (1) *M. asiaticum* 7.7 (1) *M. fortuitum* 7.7 (1)	Retrospective study on culture isolates. Clinical relevance of isolated NTM not determined. Gene sequencing not used for speciation. Treatment details including outcomes not provided.
Maurya *et al*[64], Lucknow, Uttar Pradesh Hospital-based prospective study (2015) EPTB suspects only=756 HIV status: Not available	ZN staining Liquid culture (BacT/ALERT 3D) ICA (SD MPT64 TB Ag Kit) Biochemical tests LPA (CM/AS Kit) Prevalence: 8.2% (62/756) in EPTB suspects	*M. fortuitum* 27.5 (17) *M. intracellulare* 20.9 (13) *M. abscessus* 14.6 (9) *M. chelonae* 12.9 (8) Others: MAC 8.1 (5), *M. kansasii* 4.8 (3), *M. gordonae* 3.2 (2), *M. interjectum* 3.2 (2) and other species 4.8 (3)	Biased selection of population (EPTB suspects). Molecular techniques such as gene amplification and gene sequencing not used for NTM speciation. Treatment details including outcomes not provided.

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Study details	Methods of NTM detection and identification and results	Identified NTM species	Limitations
Umrao *et al*[65], Lucknow, Uttar Pradesh Hospital-based prospective study (2013-2015) TB suspects=4,620 HIV status: Available	ZN staining Liquid culture (BacT/ALERT 3D) ICA (SD MPT64TB Ag Kit) Biochemical tests LPA (CM/AS Kit) Prevalence: 4.52% (263/4620) in TB suspects Pulmonary NTM: 79.1% (208/263) Extrapulmonary NTM: 20.9% (55/263) Other results: Three NTM patients were HIV positive	*M. abscessus* 31.3 (82) *M. fortuitum* 22 (59) *M. intracellulare* 13.6 (36) *M. chelonae* 9.1 (24) *M. avium* 7.2 (19) *M. interjectum* 3.4 (9) *M. simiae* 3.4 (9) Others: *M. gordonae* 2.6 (7), *M. scrofulaceum* 1.9 (5), *M. kansasii* 1.9 (5), *M. szulgai* 1.7 (4), *M. malmoense* 0.7 (2), *M. intermedium* 0.7 (2)	Gene sequencing not performed for NTM speciation. HIV status of the patients not provided. Clinical data of the patients not available. Treatment details including outcomes not provided.
Sairam *et al*[66], New Delhi Hospital-based retrospective study (2015-2017) Total TB suspects=877 HIV status: Not available	ZN staining GeneXpert MTB/RIF Culture Prevalence: 3.87% (34/877) in TB suspects Pulmonary NTM: 56% (19/877) Extrapulmonary NTM: 34% (15/877)	*M. intracellulare* 23.5 (8) *M. kansasii* 20.5 (7) *M. abscessus* 14.7 (5) *M. fortuitum* 2.9 (1) *M. chelonae* 2.9 (1) *M. interjectum* 2.9 (1) Other include 11 isolates	Details of methods of species identification not mentioned. HIV status of patients not provided. Data related to patients having actual disease not clear. Methods of NTM identification and speciation not clearly provided. Treatment details including outcomes not provided.
Sharma *et al*[61], New Delhi Hospital-based prospective study (2014-2017) Total TB suspects=5,409 PTB=3,840 EPTB=1,569 HIV status: Available	ZN and fluorochrome staining GeneXpert MTB/RIF Liquid culture (MGIT 960) ICA (SD MPT64TB Ag Kit) PNB-LJ culture LPA (CM/AS Kit) Mycolic acid analysis by HPLC 16S-23S rRNA ITS gene sequencing Prevalence: 0.7%(42/5409) Other results: Pulmonary NTM: 34 (81%) Extrapulmonary NTM: 8 (19%) *M. simiae* was repeatedly isolated in one patient with bronchial asthma, he was not treated because of absence of symptoms. One extrapulmonary NTM patient was HIV positive.	**Pulmonary NTM** *M. intracellulare* 32.3 (11) *M. abscessus* 26.5 (9) *M. simiae* 14.7 (5) *M. kansasii* 11.8 (4) *M. gordonae* 8.8 (3) *M. chimaera* 2.9 (1) *M. senegalense* 2.9 (1) **Extrapulmonary NTM** *M. abscessus* 75 (6) *M. intracellulare* 12.5 (1) *M. parascrofulaceum* 12.5 (1)	Multi-locus gene sequencing not performed to identify NTM to the subspecies level.

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Study details	Methods of NTM detection and identification and results	Identified NTM species	Limitations
		South zone	
Paramasivan *et al*[62], Thiruvallur, Tambaram, Madras city, Bangalore Community-based prospective study (1980-81) PTB suspects Thiruvallur: n=16,907 Tambaram: n=3,576 Madras city: n=24,121 Bangalore: n=12,909 HIV status: Pre-HIV era in India	Solid culture (LJ medium) Biochemical tests Prevalence: 8.6% (1457/16,907): Thiruvallur 7.6% (270/3576): *Tambaram* 4.5% (1095/24,121): *Madras* city 4.5% (587/12,909): Bangalore	Speciation for 1000 isolates from Thiruvallur was done *M. avium/intracellulare* 22.6 (226) *M. terrae* complex 12.5 (125) *M. scrofulaceum* 10.5 (105) *M. fortuitum* 7.6 (76) Others: *M. flavescens* 6.7 (67), *M. gordonae* 6.6 (66), *M. chelonae* 5.5 (55), *M. vaccae* 5.4 (54), *M. phlei* 3.4 (34), *M. triviale* 3.3 (33), *M. smegmatis* 1.9 (19), *M. gastri* 1.8 (18), *M. asiaticum* 1.5 (15), *M. toakiense* 1.1 (11), *M. marinum* 1 (10), *M. malmoense* 0.9 (9), *M. kansasii* 0.7 (7), *M. szulgai* 0.7 (7), *M. haemophilum* 0.6 (6), *M. xenopi* 0.5 (5), *M. ulcerans* 0.5 (5), *M. aurum* 0.5 (5), *M. thermoresistable* 0.2 (2), *M. aichiense* 0.2 (2), *M. simiae* 0.1 (1), *M. thermophilum* 0.1 (1), *M. neoaurum* 0.1 (1)	Study done in pre-HIV era in India, therefore, it may not provide the true prevalence of NTM disease in the region.
Jesudason and Gladstone[67], Vellore, Tamil Nadu Hospital-based prospective study (1999-2004) Total TB suspects=32,084 HIV status: Available	ZN staining, Solid culture (LJ medium) Biochemical tests DST for rapidly growing NTM on Mueller-Hinton agar and for slow growing NTM on LJ medium was done Prevalence: 0.5% (173/32,084) among TB suspects Other results: Pulmonary NTM: 9.8% (17/173) Extrapulmonary NTM: 90.2% (156/173) 6 NTM patients were HIV positive	Speciation was done only in 115 isolates *M. chelonae* 46 (53) *M. fortuitum* 41 (47) *M. szulgai* 2.6 (3) *M. terrae* 2.6 (3) Others: *M. smegmatis* 1.73 (2), *M. scrofulaceum* 0.9 (1), *M. simiae* 0.9 (1), *M. flavescens* 0.9 (1) and *M. gordonae* 0.9 (1)	For NTM identification, newer molecular techniques such as gene probes, PCR and DNA sequencing not used. Clinical significance of isolated NTM not established. Data for pulmonary and extra-pulmonary NTM disease provided only for 115 patients. Treatment details including outcomes not provided.
Sivasankari *et al*[68], Puducherry Hospital-based prospective study (2003-2004) Total TB suspects=635 PTB=337 EPTB=298 HIV status: Available	ZN and fluorochrome staining Culture LJ medium Biochemical tests Prevalence: 0.8% (5/635) Other results: All patients had extrapulmonary NTM disease	*M. kansasii* 60 (3) *M. flavescens* 20 (1) *M. gordonae* 20 (1)	Molecular techniques such as HPLC, gene amplification and gene sequencing not used. Treatment details including outcomes not provided.

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Study details	Methods of NTM detection and identification and results	Identified NTM species	Limitations
Radha Bai Prabhu *et al*[69], Kancheepuram, Tamil Nadu Hospital-based prospective study (2008-2016) TB suspected tubal disease females=173 Only extrapulmonary specimens=urine, POD fluid and endometrial samples HIV status: Available	ZN and fluorochrome staining Liquid culture (MGIT 960) Histopathological examination PCR Mycolic acid analysis by HPLC Prevalence: 23.7% (63/173) among tubal disease suspects Other results: 41 NTM isolates were associated with tubal disease	*M. chelonae* 25.4 (16) *M. fortuitum* 6.3 (4) *M. simiae* 3.2 (2) *M. kansasii* 1.6 (1) *M. intracellulare* 1.6 (1) *M. marinum* 1.6 (1)	Biased selection of population. Molecular methods for species identification not used Clinical relevance of isolated NTM species was not established. Treatment details including outcomes not provided.
	West zone		
Narang *et al*[70], Wardha, Maharashtra Hospital-based prospective study (2001-2002) HIV-TB coinfection suspects=71 PTB=53 EPTB=14 (In 4 patients, information regarding pulmonary and extrapulmonary status was not available) HIV status: All positive	Liquid culture (BACTEC 460TB) Biochemical tests Mycolic acid analysis by HPLC Prevalence: 8.4% (6/71) in HIV-TB suspected patients Other result Extrapulmonary NTM=6	MAC 50 (3) *M. simiae* 50 (3)	Biased selection of the study population (HIV patients only). Molecular techniques not used for species identification. Clinical relevance of isolated NTM not discussed. Treatment details including outcomes not provided.
Shenai *et al*[71], Mumbai, Maharashtra Hospital-based prospective study (2005-2008) Total TB suspects=14,627 HIV status: Available	Liquid culture (MGIT 960) PNB-LJ culture NAP test (BACTEC 460 TB) RLBH assay of *rpoB* gene PCR-RE assay and gene sequencing Prevalence: 0.8% (127/14627) Other results: Pulmonary NTM: 81% (103/127) Extrapulmonary NTM: 19% (24/127) three NTM cases were HIV positive	*M. intracellulare* 40 (32), *M. simiae* 35 (28), *M. abscessus* 59 (27), *M. fortuitum* 29 (19), *M. kansasii* 6 (5), *M. gordonae* 4 (3), *M. szulgai* 2 (2), *M. avium* 1 (1) Ten cases had mixed infection, 6 with *Mtb* and 4 had *M. kansasii* + *M. fortuitum* 1 (1), *M. avium* + *M. kansasii* 2 (2) and *M. intracellulare* + *M. gordonae* 1 (1)	Sequencing of *rpoB* gene may lead to misidentification of NTM species. Multilocus gene sequencing would have given strength to the study. Treatment details including outcomes not provided.
Goswami *et al*[63], Wardha, Maharashtra Community-based prospective survey (2007-2009) PTB suspects=6,445 HIV status: Not available	Culture Biochemical tests DST by micro-broth dilution method Prevalence: 1% (65/6445)	*M. fortuitum* 32.3 (21), *M. gordonae* 21.5 (14), *M. avium* 13.8 (9), *M. flavescens* 10.7 (7) Others: *M. scrofulaceum* 6.1 (4), *M. chelonae* 4.61 (3), *M. abscessus* 4.61 (3), *M. kansasii* 1.5 (1), *M. simiae* 1.5 (1), *M. gastri* 1.5 (1) and *M. triviale* 1.5 (1)	Study was performed in PTB suspects only. How TB and NTM were distinguished not clear. HIV status of the patients not available. Gene sequencing for speciation not performed. Patients' data not available. Treatment details including outcomes not provided.

PTB, pulmonary TB; EPTB, extra PTB; LJ medium, Löwenstein-Jensen medium; ZN staining, Ziehl-Neelsen staining; DST, drug susceptibility testing; MGIT, mycobacteria growth indicator tube; PNB, *p*-nitrobenzoic acid; NAP, *p*-nitro-alpha-acetylamino-beta-hydroxypropiophenone; RLBH, reverse line blot hybridization; PCR-RE assay, polymerase chain reaction-restriction endonuclease assay; ICA, immunochromatographic assay; MPT64, mycobacterial protein 64 KD; LPA, line probe assay; 16S-23S rRNA ITS sequence, 16S-23S ribosomal RNA internal transcribed spacer sequence; MAC, *Mycobacterium avium* complex; POD, pouch of douglas; HPLC, high-performance liquid chromatography

in study designs, (*ii*) standard American Thoracic Society (ATS) (2007)[17] and British Thoracic Society (BTS) (2017)[1] guidelines criteria were not followed in most of these studies, (*iii*) only laboratory-related NTM culture data have been reported, and (*iv*) most of the studies have not provided clinical details and treatment outcomes. Of the 13 studies, only two[61,71] followed ATS guidelines (2007)[17] and one of these reported treatment outcomes[61] . Future studies should report about extrapulmonary NTM diseases in addition to clinical details including treatment outcomes of various NTM diseases.

Risk factors for NTM disease

Risk factors for NTM diseases vary according to the clinical type of NTM disease[72-74]. Various risk factors for NTM-PD are described in Box IIA[72-74]. Pre-existent lung disease is mostly present in these patients. In the absence of obvious structural lung disease, patients may have quantitatively impaired ciliary function or may be heterozygous for cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations[75,76]. Extrapulmonary NTM disease can occur due to breaches in skin or soft tissues or due to several nosocomial factors, which are detailed in Box IIB[72-74]. Disseminated NTM disease generally occurs in patients having primary or acquired immunodeficiency conditions. Certain environmental and organism-related factors such as water sources and reservoirs, and NTM growth characteristics in different climatic conditions, have also been reported as risk factors (Box IIB)[72-74]. In addition, habits, hobbies and profession of an individual may also increase the risk of having NTM disease[74].

Immunopathogenesis of NTM disease

In addition to lung, the most common organ involved affected by NTM, localized and disseminated NTM infections can occur[73]. Patients with disseminated NTM infections (defined as involvement of two or more non-contiguous body organs) usually have underlying generalized immune defect such as HIV/AIDS, and 2-8 per cent of these patients may have concurrent pulmonary involvement[77]. Identification of the underlying immune defect is crucial for early diagnosis, treatment and prevention. Patients with NTM disease and underlying primary immunodeficiencies typically present in their childhood or adulthood, whereas those with acquired immunodeficiencies can present at any age (Table VI)[73].

Antimycobacterial cell-mediated immunity requires a close interaction between myeloid and lymphoid cells (Fig. 1)[73]. Mononuclear phagocytes after engulfing mycobacteria secrete interleukin-12 (IL-12) which, in turn, stimulates T cells and NK (natural killer) cells through the IL-12 receptor (heterodimer of IL12RB1 and IL12RB2). A complex cascade is triggered by IL-12 receptors *via* TYK2 (tyrosine kinase) and JAK2 (Janus kinase) signals, leading to STAT-4 (signal transducer and activator of transcription) phosphorylation, homodimerization and nuclear translocation to induce interferon-gamma (IFN-γ) secretion (Fig. 1). IFN-γ binds to its receptor IFNG receptor (IFNGR) (heterodimer of IFNGR1 and IFNGR2) and leads to phosphorylation of JAK2, JAK1 and STAT1 and phosphorylated STAT1 (pSTAT1) homodimerisation. The pSTAT1 homodimer [IFN-γ activators (GAF)] binds to IFN-γ activation sequence which upregulates IFN-γ responsive gene transcription. This cascade leads to activation and differentiation of macrophages. As a result, upregulation of IL-12 and tumour necrosis factor-α (TNF-α) secretions facilitates granuloma formation. After these events, macrophages can kill intracellular mycobacteria being assisted by maturation of mycobacterial phagosome, nutrition deprivation and induction of autophagy, exposure to antimicrobial peptides and reactive oxygen species. The nuclear factor (NF) κB essential modulator-mediated pathway and oxidative burst from macrophages are also important to fight against NTM infection[73]. Genetic defects in any of these immune factors may disturb the cascade of protection against mycobacterial infection and may lead to disseminated NTM disease[73]. These immune defects have been summarized in Table VI[73].

Clinical manifestations

The clinical manifestations of NTM disease are similar to those of TB and may pose a diagnostic challenge even to an experienced clinician. NTM disease is classified into four clinical types: (*i*) chronic PD, (*ii*) lymphadenopathy, (*iii*) skin and soft tissues, rarely, bones and joints, and (*iv*) disseminated disease[73].

Chronic pulmonary disease (PD)

The ATS and Infectious Disease Society of America (IDSA), 2007[17], and BTS, 2017[1], ATS/ERS/ESCMID/IDSA[18] have published guidelines to standardize the diagnosis and treatment of NTM diseases. While evaluating NTM suspects, the following criteria

Box II. (A and B): Risk factors for nontuberculous mycobacterial disease

(A) Risk factors based on disease sites

Pulmonary NTM disease	Extrapulmonary NTM disease (generally related to healthcare and commercial establishments)
Destroyed lungs due to TB or other diseases like pneumoconioses	Trauma (direct infection from environs)
Bronchiectasis (*esp*. middle lobe and lingula) due to any cause	Cosmetic surgeries
Chronic obstructive pulmonary disease	Prosthetic devices and implants
Cystic fibrosis-CFTR gene polymorphism*	Organ transplantation
Primary ciliary dyskinesia	Dental procedures and surgeries
Alpha 1 antitrypsin deficiency	Intramuscular or intradermal injection
Lung cancer	Joint injections
Thoracic skeletal abnormalities (kyphoscoliosis)	Invasive devices (*e.g.,* pacemakers)
Lady Windermere syndrome†	Medical tourism (individuals infected with NTM visiting to some other country)
Gastroesophageal reflux disease‡	
Pulmonary alveolar proteinosis	
Rheumatoid arthritis with lung involvement	

*NTM are isolated in sputum cultures of 3-19.5% of CF patients (majority are MAC). †High prevalence (26-44%) of NTM disease especially nodular-bronchiectatic type in nonsmoking postmenopausal white women who are taller and lean with scoliosis, pectus excavatum and mitral valve prolapse syndrome than their peers, ‡In gastroesophageal reflux disorders, RGM are commonly involved in the disease such as *M. fortuitum*. BMI: body mass index; CFTR: cystic fibrosis transmembrane receptor; MAC, *Mycobacterium avium* complex; CF, cystic fibrosis

(B) Miscellaneous risk factors

(*i*) Immunodeficiency states

(a) Primary*	(b) Acquired
Anti-interferon γ-antibodies (blocking of interferon γ-interleukin-12 pathway)	HIV/AIDS status (CD4 counts <50 cells/µl)
Anti GM-CSF antibodies (impaired local immunity)	Use of biologics (anti-TNF agents and TNF receptor blockers)
NEMO mutations (impaired signal transduction from Toll-like receptors, interleukin-1, and TNFα)	Use of immunosuppressive agents and steroids
STAT1 deficiency (low systemic immunity)	
IL12 mutations (reduced T-cells and natural killer cells stimulation)	
CYBB mutations (decreased bactericidal activity)	
GATA2 gene mutations (impaired hematopoietic, lymphatic, and vascular development)	

(*ii*) Environmental factors

(a) Household and lifestyle factors	(b) Climatic and bacterial population factors
Soil exposure	Larger water surface area
Showers and hot tubs	Higher mean daily potential evapotranspiration
Municipal water supply	Higher copper soil levels (helps mycobacteria to form biofilms)
Kitchen sink biofilms, ice machines, refrigerator taps	Higher sodium soil levels (more nutrition for mycobacteria)
Indoor swimming pool use in past 4 months	Lower manganese soil levels (manganese inhibits mycobacterial growth)
Outdoor swimming pool use for at least once a month	Lower top soil depth (high nutrition for mycobacteria due to low vegetation)
Infection from spa, Jacuzzi, whirlpool footbath, saunas, pedicure procedures	

*These mutations are rare and associated with disseminated NTM disease. GM- CSF, granulocyte macrophage colony stimulating factor; NEMO, nuclear factor κB essential modulator; STAT1, Signal transducer and activator of transcription 1 (for disseminated infection); IL-12, interleukin-12; TNF, tumor necrosis factor; CYBB, cytochrome b-245 beta. *Source*: Refs 72-74

Table VI. Primary and acquired immune deficiencies associated with disseminated non-tuberculous mycobacterial (NTM) infection

Immunodeficiency	Inheritance	Disease onset	BCG infection	Systematic *Salmonella* infection	Other possible infection	Granuloma formation	Response to antimicrobial	Indication for immunotherapy	Prognosis
Early onset									
IFNGR1/R2									
Complete	AR	Infancy/early childhood	Yes	Yes	Listeriosis, herpes virus, respiratory syncytial virus, parainfluenza virus infections, TB	No	Very poor	No	Poor
Partial	AR	Late childhood	Yes	Yes	TB	No report	Favourable	Variable	Good
Partial	AR	Late childhood/ adolescence	Yes	Yes	Histoplasmosis, TB	Yes	Favourable	Yes	Good
IL12B	AR	Infancy/early childhood	Yes (97%)	Yes (25%)	CMC, disseminated TB, nocardia, *Klebsiella* spp. infection	Yes	Favourable	Yes	Fair
IL12RB1	AR	Early childhood	Yes (76%)	Yes (43%)	TB, CMC (24%), *Klebsiella* spp. infection	Yes	Favourable	Yes	Fair
STAT1 LOF									
Complete	AR	Infancy (die early without HSCT)	Yes	No	TB, fulminant viral infection (mainly herpes)	Yes	Poor	No	Poor
Partial	AR	Infancy/early childhood/ adolescence	Yes	Yes (50%)	Severe, curable viral infection (mainly herpes)	No report	Favourable	Yes	Fair
Partial	AD	Infancy/early/ childhood/ adolescence	Yes	No	TB	Yes	Favourable	Yes	Good
IRF8	AR	Infancy	Yes	No	CMC	Poorly formed	Poor	No	Poor
IRF8	AD	Late infancy	Yes	No	No report	Yes	Favourable	No	Good
ISG15	AR	Infancy	Yes	Yes	No report	No report	Favourable	Yes	Good
NEMO	XR	Early to late childhood	Yes	No	Invasive Hib infection TB	Yes	Variable	Yes	Fair
CYBB	XR	Infancy/early childhood	Yes	No	TB	Yes	Fair	No	Fair

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Immunodeficiency	Inheritance	Disease onset	BCG infection	Systematic *Salmonella* infection	Other possible infection	Granuloma formation	Response to antimicrobial	Indication for immunotherapy	Prognosis
Late onset									
GATA2	AD	Late childhood/ adulthood	No	No	HPV, CMV, EBV, *Clostridium difficile* infections, histoplasmosis, aspergillosis	Yes	Poor	Yes	Poor
Anti-IFN-γ antibodies	Acquired	Young adult to elderly	No	Yes	*Salmonella* spp., *Penicillium* spp., *Histoplasma* spp., *Cryptococcus* spp., *B. pseudomallei*, VZV, CMV infections	Yes	Poor	No	Fair

AR, autosomal recessive; AD, autosomal dominant; CMC, chronic mucocutaneous candidiasis; LOF, loss of function; HSCT, haemopoietic stem cell transplantation; Hib, *Haemophilis influenzae* type b, HPV, human papillomavirus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; VZV, varicella zoster virus; BCG, bacille Calmette-Guerin; *B. pseudomallei, Burkholderia pseudomallei*; IFN-γ, interferon-gamma; XR, X-linked recessive; IFNGR, interferon-gamma recapter; IL, interleukin; STAT, signal transducer and activator of transcription; IRF, interferon regulatory factor; ISG, interferon-stimulated genes; NEMO, nuclear factor kappa-light-chain-enhancer of activated B cells essential modulator; GATA, transcription factor implicated in early hematopoietic, lymphatic and vascular development. Note: Investigations for GATA2 deficiency should be done in patients with myelodysplastic syndrome and mycobacterial disease. *Source:* Reproduced with permission from Ref. 73

should be followed: (*i*) pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or high-resolution computed tomography (CT) scan that shows multifocal bronchiectasis with multiple, small nodules; (*ii*) positive culture results from at least two separate expectorated sputum samples [if the results from the initial sputum samples are non-diagnostic, consider repeat sputum acid-fast bacilli (AFB) smear and culture]; single-positive NTM culture from CT-directed bronchoalveolar lavage or bronchial washing specimen from the affected lung segment of NTM suspect who cannot expectorate sputum or whose sputum is consistently culture-negative; and (*iii*) other disorders such as TB and fungal infections must be excluded[1,17].

Patterns of NTM-PD: Chronic PD is the most common form of NTM disease. Three patterns of pulmonary involvement have been described[17]: (*i*) fibro-cavitary type, which usually occurs in the upper lobe with a history of smoking in an older male patient with pre-existent lung disease such as chronic obstructive pulmonary disease (COPD), bronchiectasis and CF (Fig. 2); (*ii*) nodular/bronchiectatic type of pattern occurring in post-menopausal, non-smoking females, predominantly having right middle lobe and left lingular bronchiectasis with a few lung nodules. This syndrome was described after the main character in Oscar Wilde's eponymous play as 'Lady Windermere syndrome'[78], and was believed to occur from voluntary cough suppression[79], however, subsequently, this hypothesis was discarded[80]. Other features include mitral valve prolapse, scoliosis and pectus excavatum; high prevalence of gastro-oesophageal reflux disease (GERD) (26-44%)[81,82]; increased adiponectin; decreased leptin and estrogen levels and abnormalities in fibrillin gene. Presence of all these features increases the susceptibility of these females to MAC infections[83]; and (*iii*) hypersensitivity pneumonitis-like NTM PD or 'hot tub lung' occurring due to exposure to aerosols from indoor hot tub. Various risk factors for NTM-PD are listed in Box IIA.

NTM species and NTM-PD: Because of variable virulence, it is important to identify NTM species and *M. abscessus* subspecies for the management of NTM-PD. It has been reported that only 25-60 per cent of patients with positive respiratory specimen fulfil clinical, radiographic and microbiological criteria of NTM-PD[84]. Patients in whom *M. kansasii* and *M. malmoense* are isolated from respiratory specimens frequently meet clinical disease criteria, as these

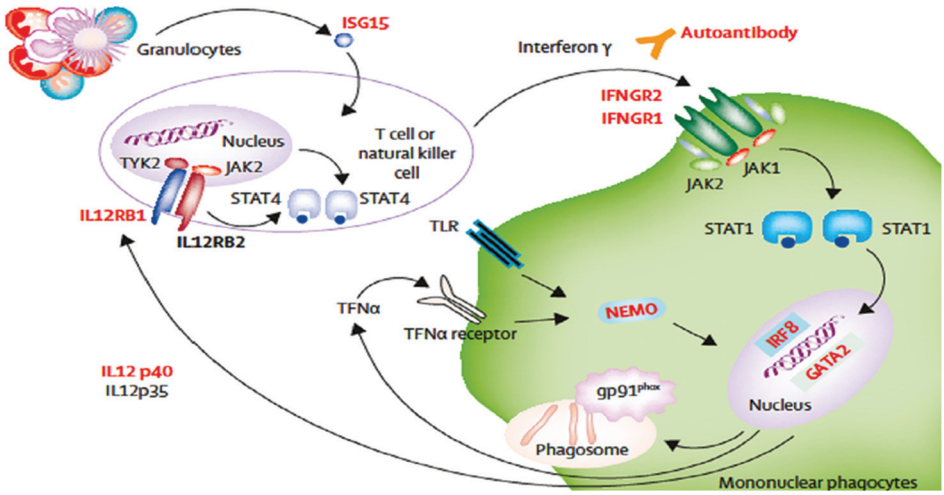

Fig. 1. Host defence mechanisms against non-tuberculous mycobacteria (NTM). Defects leading to disseminated NTM infection are shown in red. ISG15, interferon-stimulated gene 15; IFNGR, interferon-gamma receptor; TYK, tyrosine kinase; JAK, Janus kinase; STAT, signal transducer and activator of transcription; IRF, interferon regulatory factor; GATA, transcription factor implicated in early haemopoietic, lymphatic, and vascular development; NEMO, nuclear factor kappa-light-chain-enhancer of activated B cells essential modulator; IL, interleukin; TNF, tumour necrosis factor; TLR, toll-like receptors. *Source*: Reproduced with permission from Ref. 73.

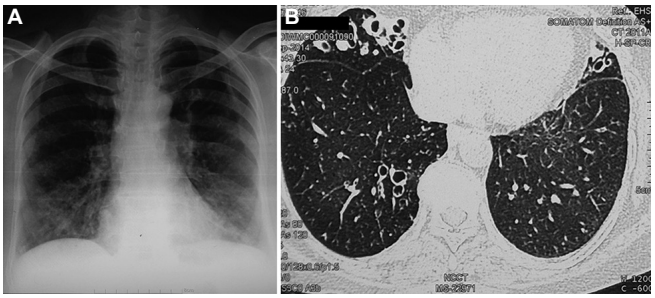

Fig. 2. (A) Chest radiograph in a 62 yr old female with asthma, allergic bronchopulmonary aspergillosis and bronchiectasis. *Mycobacterium simiae* was isolated repeatedly from the sputum. **(B)** High-resolution computed tomography chest (axial section) showing bilateral bronchiectasis in the right middle lobe, lingula and lower lobes.

NTM isolates are clinically highly relevant, whereas 40-60 per cent with MAC, *M. abscessus* and *M. xenopi* and <20 per cent of patients with *M. chelonae* and *M. fortuitum* meet clinical disease criteria[44,85-89].

The potential to produce specific clinical type of lung disease also varies among NTM species. While *M. kansasii*, *M. xenopi* and *M. malmoense* commonly cause fibro-cavitary disease but rarely nodular-bronchiectatic disease[17,44,90,91], MAC and *M. abscessus* cause both types of NTM-PDs and MAC and *M. immunogenum* cause hypersensitivity pneumonitis-like NTM-PD[17].

MAC is the most common NTM isolated from respiratory secretions in patients with NTM-PD. While a single strain of MAC species is repeatedly isolated in the cavitary type, several strains of MAC species may occur simultaneously or the strain may change sequentially in nodular-bronchiectatic type[92,93]. Relapse versus new re-infection of MAC infection after treatment completion can be differentiated by MAC genotyping[94].

According to one study from the USA, while tap water was the source of *M. avium* infection, soil was the source of *M. intracellulare* infection[94]. It has been suggested that patients with *M. intracellulare* lung disease present at a later stage with adverse prognosis than patients with *M. avium* lung disease, and *M. chimaera* is less virulent than *M. avium* and *M. intracellulare*[95,96]. Significant geographic variation exists in the distribution of NTM species in the USA; where *M. avium* complex was the most common species isolated in the South, *M. abscessus*/*M. chelonae* was proportionately higher in the West in one study[97]. MAC species also vary from region to region: while *M. avium* is dominantly found in South America and Europe, *M. intracellulare* is found in South Africa and Australia[23]. Recurrence rates in MAC-associated lung disease also differ among MAC species[95].

The second common NTM species also has a geographical variation. While *M. abscessus* is the second most common cause of NTM-PD in the USA[98], *M. kansasii* in some European countries including the UK, *M. xenopi* in some parts of Europe and Canada

and *M. malmoense* in northern Europe are the second most common causes of NTM-PD[99]. *M. kansasii*, one of the slowly growing NTM, is most virulent[98]. About 80 per cent of NTM-PD due to RGM results from *M. abscessus*[100]. There are three subspecies of *M. abscessus*: (*i*) *M. abscessus* subsp. *abscessus*, which is the most common pathogen (45-65%), followed by (*ii*) *M. abscessus* subsp. *massiliense* (20-55%), and (*iii*) *M. abscessus* subsp. *bolletii* (1-18%)[101]. Patients with gastro-oesophageal disease may have NTM-PD due to RGM such as *M. fortuitum*[17].

Clinical features: Respiratory symptoms and signs in NTM-PD vary depending on the clinical type. In the cavitary type, these may be severe due to the pre-existent underlying lung disease and include shortness of breath, cough with expectoration and haemoptysis, whereas patients with nodular-bronchiectasis have milder respiratory symptoms without pre-existing parenchymal lung disease and nagging cough may be prominent. Constitutional symptoms such as fever, anorexia, progressive fatigue, malaise and weight loss may be present especially in cavitary type of NTM-PD[1,17]. The clinical and radiographic presentation in *M. kansasii* PD is similar to *Mtb* and includes fever, cough with or without haemoptysis and chest pain, and chest X-ray often shows infiltrates and cavitary lesions[17,102] (Fig. 3). Patients with hypersensitivity pneumonitis-like NTM-PD have subacute onset of respiratory symptoms involving young individuals without pre-existing lung disease and the prognosis is good[17,103,104].

Lymphadenitis

In low TB-burden countries, single-site lymphadenitis is the most common manifestation of NTM infection in younger children[74,105]. Solitary lymph node is usually localized to the submandibular or cervical region and rarely, can also involve other groups either singly or multiple such as axillary, inguinal region in the disseminated NTM disease in severely immunocompromised individuals[106]. The lymph node enlargement usually starts as a painless swelling and later in the advanced stage, the swelling becomes fluctuant with pus inside, which may later burst out with a sinus formation. Constitutional symptoms such as fever, weight loss and fatigue may be absent. Smear microscopy and culture may be negative because of paucibacillary nature of the disease[17]. Molecular tests may be used to establish the diagnosis. MAC is the most frequently isolated NTM species. There is an inverse relationship of TB incidence and NTM disease and in high TB-burden countries, *Mtb* is the most frequent cause of lymphadenitis in all ages[106].

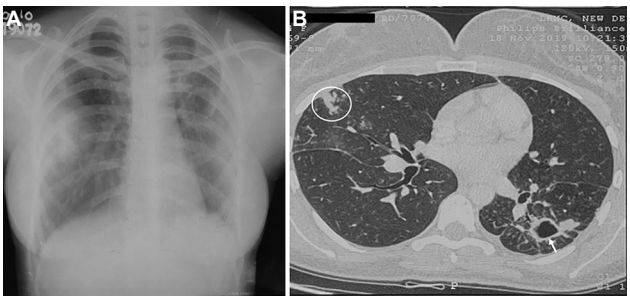

Fig. 3. Chest radiograph in a 29 yr old female patient with *Mycobacterium kansasii*-pulmonary disease. (**A**) Chest X-ray reveals a cavitary lesion in the left lung. (**B**) Axial section in the high-resolution computed tomography scan demonstrates a cavity in the left lung (white arrow) and tree-in-bud appearance in the right lung (white circle).

Skin, soft tissues and bone NTM infections

Three types of clinical presentations have been described: (*i*) Buruli ulcer (predominantly occurring in Uganda) or Bairnsdale ulcer disease (predominantly occurring in Australia), certain regional pockets in Latin America and China: it is a severe cutaneous disease due to *M. ulcerans* which progresses from nodular cutaneous lesions into large painless ulcers[107]. These organisms produce a toxin, mycolactone, which produces damage to the skin[108]. Early diagnosis and treatment is essential to minimize morbidity and costs and prevent long-term disability[109]; (*ii*) infection due to *M. marinum* is also known as fish-tank granuloma (previously known as swimming pool granuloma) and the infection can be acquired from swimming pools, cleaning of fish tanks or any other fish- or water-related activity[110]. Organisms usually gain access through skin cuts or abrasions[111]. It starts as a single papulonodular, verrucous or ulcerated granulomatous lesion over the hand and forearm that progresses to form multiple skin lesions in a sporotrichoid pattern - appearance which is similar to skin lesions due to *Sporothrix schenckii* and rarely, the underlying bone involvement occurs[112]; and (*iii*) localized skin and soft-tissue infections occurring due to RGM (*M. abscessus, M. fortuitum* and *M. chelonae*) at wound or injection sites[113-115] (Figs 4 and 5) and slowly growing mycobacteria in both immunocompromised and immunocompetent individuals[115,116]. These organisms gain access through skin breaks following trauma and surgical procedures, following the use of surgical instruments without autoclaving, during cosmetic surgery, pedicure and manicure procedures in beauty salons, surgical

procedures involving placement of various implants, in mesh used for hernial site repair (Fig. 6), tattooing procedures following inoculation of contaminated ink containing *M. haemophilum*, intravenous punctures and lines, abscesses due to intramuscular injections through contaminated needles and use of tap water for skin cleaning[112,113].

Disseminated NTM disease

Disseminated NTM disease due to MAC is frequent in HIV/AIDS especially in patients with CD4+ lymphocyte count <50 cells/µl. Isolated pulmonary involvement is rare in HIV/AIDS[117]. Pulmonary involvement occurs in 2.5-8 per cent of patients with disseminated MAC[77]. The portal of entry in these patients is believed to be through bowel[118-120] and occasionally through lungs with subsequent haematogenous dissemination. MAC (predominantly *M. avium*) is the most common NTM species isolated in these patients[17]. These patients typically present with insidious onset of constitutional symptoms comprising fever with night sweats, weight loss, abdominal pain, diarrhoea and malaise[17]. They may have anaemia, hepatosplenomegaly and lymphadenopathy[17]. Somehow, disseminated NTM infections due to rapidly growing NTM (*M. abscessus* and *M. fortuitum*) are rare in HIV/AIDS patients[121]. Besides *M. avium*, less common NTM species such as *M. genavense* and *M. simiae* can also cause disseminated NTM disease in HIV/AIDS patients[17].

M. kansasii can cause pulmonary involvement in HIV/AIDS patients at higher CD4+ counts, and its isolation should always be considered a potential pathogen[17,122]. Pulmonary involvement can also occur in other immunocompromised populations such as organ transplantation (6.5%)[123], bone marrow (2.9%)[124] and rarely liver and kidney transplantation. CF patients undergoing lung transplantation may develop life-threatening infection with *M. abscessus*[124]. Disseminated NTM infections can also occur in a few other rare settings (Fig. 7A-G) which will require appropriate investigations. These have been listed in Box IIB[73]. NTM, especially *M. abscessus* (Fig. 7) and *M. fortuitum,* may infect deep indwelling lines[17,122]. Anti-tumour necrosis factor-α agents (infliximab, etanercept and adalimumab) used to treat several diseases such as rheumatoid arthritis, psoriatic arthritis and inflammatory bowel disease can predispose to both TB and NTM diseases[125]. A good response to rituximab in disseminated MAC patients with interferon-gamma autoantibodies has also been reported[126,127].

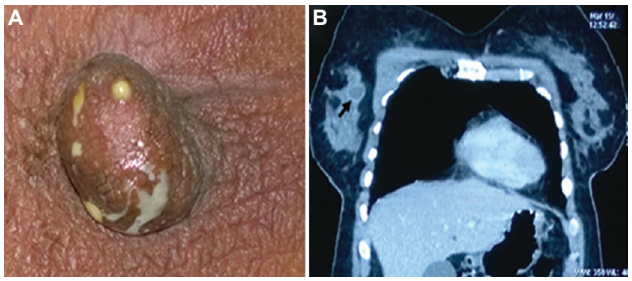

Fig. 4. (**A**) A 35 yr old female presented with discharge from the right nipple, *Mycobacterium abscessus* was isolated from the pus on several occasions prior to treatment. (**B**) Computed tomography (CT)-chest showing enhancement of the margin of the abscess (black arrow) with intravenous contrast. *Source*: Reproduced with permission from Ref. 61.

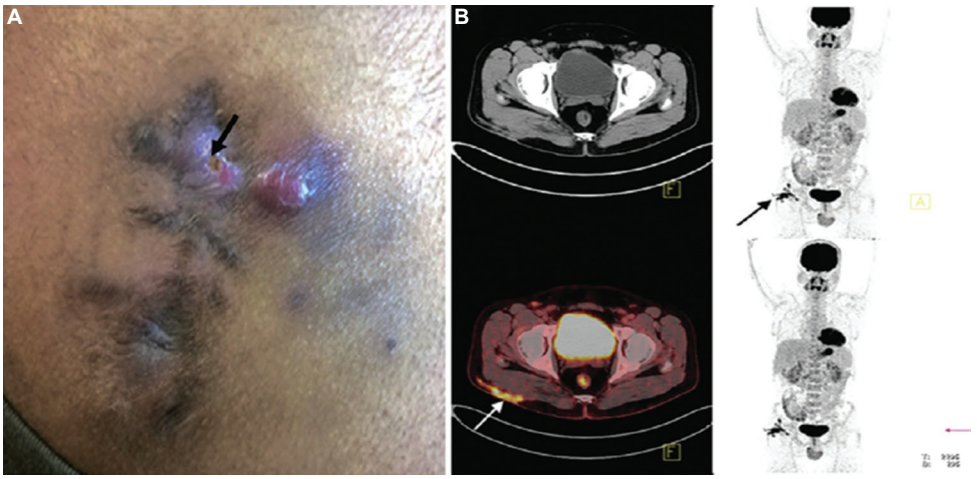

Fig. 5. (**A**) Clinical photograph of a 30 yr old male, showing right-sided post-injection gluteal abscess (black arrow) in a patient with NTM infection. (**B**) Transaxial fused ^{18}F-fluorodeoxyglucosepositron emission tomography-computed tomography (^{18}F-FDG-PET-CT) image of the same patient, at the level of acetabulum showing FDG accumulation in the subcutaneous thickening and stranding (arrow) involving the underlying right gluteus muscle superficially in right gluteal region. *Source*: Reproduced with permission from Ref. 61.

Diagnosis

Criteria for the diagnosis of NTM disease

Healthcare providers should carefully assess causality association of the isolated NTM species with patient's symptoms and signs. Approximately, one-third of NTM species are potentially pathogenic for humans[128]. Some of the common pathogenic NTM species are listed in Table VII[2,3,10,129]. It is possible that an individual with a particular NTM isolate may not have an active disease or the isolate may not be clinically relevant. While evaluating NTM suspects, the following criteria should be followed: (*i*) pulmonary symptoms, nodular or cavitary opacities on chest radiograph or high-resolution CT scan that shows multifocal bronchiectasis with multiple small nodules; (*ii*) positive culture results from at least two separate expectorated sputum samples (if the results from the initial sputum samples are non-diagnostic, consider repeat sputum AFB smear and culture; single-positive NTM culture from CT-directed bronchoalveolar lavage or bronchial washing specimen from the affected lung segment of NTM suspect who cannot expectorate sputum or whose sputum is consistently culture negative); and (*iii*) other disorders such as TB and fungal infections must be excluded[1,17].

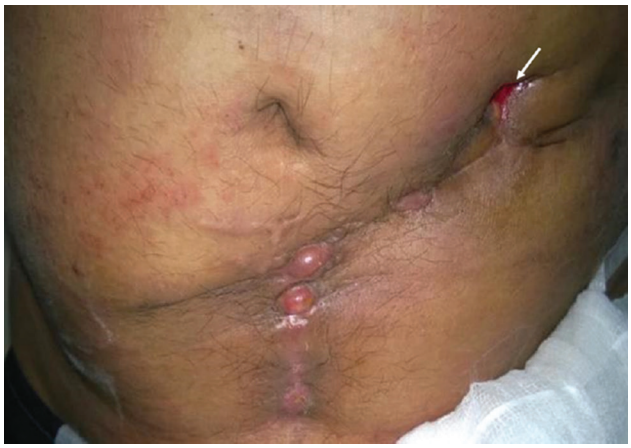

Fig. 6. Clinical photograph of a 35 yr old male, showing discharging sinus (white arrow) in the abdominal wall in a patient infected with *Mycobacterium abscessus* following hernia repair with mesh. *Source*: Reproduced with permission from Ref. 61.

Differential diagnosis

Because of similar clinical features and radiographic appearances, diseases such as TB, recurrent pulmonary aspirations, pneumonitis, bronchiectasis, histoplasmosis, aspergillosis and lung cancer should be considered in the differential diagnosis and should be appropriately ruled out. In the laboratory, the presence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*,

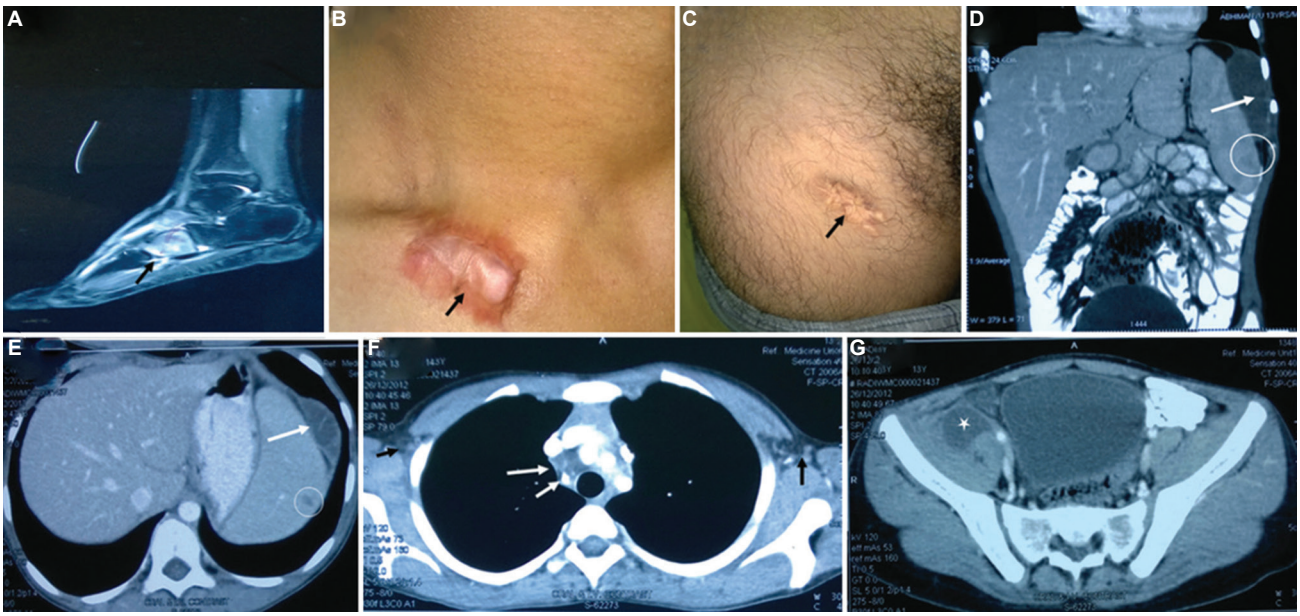

Fig. 7. The patient, a 14 yr old male, had disseminated *Mycobacterium intracellulare* infection; no immune defect could be detected. He was successfully treated. (**A**) The magnetic resonance imaging scan shows osteomyelitis of foot bone (black arrow). (**B**) Black arrow shows healing of cutaneous lesion by keloid formation. (**C**) Upper part of thigh shows another healed skin lesion (black arrow). (**D** and **E**) Hypodense lesions in the spleen (white open circles) and peri-splenic abscess (white arrows). (**F**) Bilateral conglomerate necrotic axillary (extreme-left and -right arrows) and right paratracheal lymph nodes (long and short arrows in the centre of CT image), calcification is also noted in the lymph nodes. (**G**) Iliopsoas abscess on the right side (white asterisk). *Source*: Reproduced with permission from Ref. 61.

Table VII. Clinically relevant non-tuberculous mycobacteria species

Types of disease	Names of species
Pulmonary disease	MAC, *M. kansasii, M. abscessus, M. xenopi, M. simiae, M. malmoense*
Cervico-facial lymphadenitis	*M. scrofulaceum, M. avium, M. malmoense, M. lentiflavum, M. bohemicum*
Skin and soft tissue	*M. ulcerans, M. marinum, M. abscessus, M. fortuitum, M. haemophilum, M. chelonae*
Bone and joints	MAC, *M. kansasii, M. abscessus, M. xenopi, M. goodii, M. terrae*
Disseminated disease	*M. avium, M. intracellulare, M. haemophilum, M. genavense*

MAC, *Mycobacterium avium* complex. *Source*: Refs 2, 3, 10, 129

Nocardia and *Aspergillus* in the specimens must be carefully tested[17]. It is important to consider the differential diagnosis of *Sporothrix schenckii* infection in patients suspected to have skin and soft-tissue NTM disease due to *M. marinum*[113].

Specimen collection, transportation and processing

A proper sample collection is crucial to establish a correct laboratory diagnosis of NTM disease. In case of NTM-PD patients, during collection of sputum, environmental and personal contamination should be avoided. To differentiate NTM-PD from occasional presence of NTM in tracheobronchial tract, at least 3 sputum specimens should be tested on separate occasions[18]. Sampling from extrapulmonary specimens should be obtained directly from the lesion or organ concerned[130]. Further, instruments used for sampling should be devoid of any contamination, especially in hospital settings. Storage and transportation of specimens should be done carefully[130]. Once the specimen reaches the laboratory, the process of decontamination should be done in fully sterilized set-up. As NTM are resistant to most of the common disinfectants, careful selection of disinfectants is necessary[130]. Various precautions for sample collection, transportation and laboratory processing are listed in Box III[130].

Laboratory diagnosis of NTM disease

Figure 8 illustrates various steps for NTM isolation and identification in the laboratory. Initially, the specimens are simultaneously subjected to AFB (Ziehl-Neelsen or fluorochrome) staining and GeneXpert for *Mtb* detection. Samples that are positive on AFB staining and negative on GeneXpert are considered NTM suspects, and the culture for such specimens should be done. Most of the NTM are cultivable in Lowenstein-Jensen, Middle-brook and Dubos Broth and Agar. A novel agar-based medium, RGM medium, has been specifically developed for the isolation of rapidly growing NTM. It provides an alternative method for the recovery of NTM from respiratory specimens, particularly from CF patients, by offering a simple and rapid method for specimen processing[131]. For some NTM species, additional supplements (haemin for *M. haemophilum* and mycobactin J for *M. paratuberculosis* and *M. genavense*)[130] are added in the culture medium for optimal growth. Incubation temperatures of 36±2°C for SGM and 28±2°C for RGM have been recommended[18]. Appropriate adjustments in the incubation temperature (*M. xenopi*: 42-45°C, *M. ulcerans* and *M. marinum*: 30°C) may be done for a few NTM species[18,130]. Some NTM species such as *M. tilburgii* which are not cultivable need to be tested directly from the specimen using molecular methods[132]. In patients with a high suspicion of NTM-PD but negative cultures, reassessment of decontamination procedures, use of supplemented media and molecular methods may be helpful[18]. Culture isolates of NTM-suspected specimens should be tested with *Mtb*-specific tests such as MPT64 antigen immunochromatographic test or GeneXpert, and if found negative, then it is likely to be NTM and thereafter its species identification should be done.

Earlier, several biochemical tests were done for NTM identification[130] (Table VIII). These tests were cumbersome and time consuming and are obsolete now. High-performance liquid chromatography (HPLC)-based analysis of mycolic acid was used for NTM identification in the past. This method identifies slowly growing NTM species such as MAC and *M. kansasii,* but it is less specific in identifying RGM accurately[130,133]. It also has low discriminatory power to identify closely related SGM and RGM species[130,133]. These tests have now been replaced by molecular tests for NTM species and subspecies identification. These tests include polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, gene probes and line probe assays (LPA)[130] (Table VIII).

Box III. Essentials for Identification of non-tuberculous mycobacteria (NTM)

Sample collection and transportation to the laboratory
For respiratory specimens, individuals should not rinse their mouths with tap water or other fluids before submitting the specimen.
Use a sterile, leak proof, disposable plastic container. Avoid waxed containers. Swabs are not recommended for the isolation of mycobacteria.
Collect specimens aseptically, reducing contamination with indigenous microbiota.
Collect initial specimens before antimicrobial therapy is started.
Three early morning specimens collected on three consecutive days are ideal.
For induced sputum, sterile hypertonic saline (3-5%) should be used. Avoid contamination with nebulizer reservoir water.
In case of BAL or bronchial wash, bronchoscope should be sterile, cleaned with suitable disinfectant not with tap water and saline used should be devoid of any micro-organism growth. (Lidocaine used during BAL procedure may inhibit growth of NTM).
While collection of extrapulmonary specimens, surgical instruments should be cleaned cautiously avoiding tap water or stored water. Formalin should not be used as transfer medium.
Once samples stored in container, it should not be opened until it reaches to the laboratory.
Store at 2-8°C (do not freeze) if transport is delayed more than one hour; should not be kept more than one week
Precautions in the laboratory
Effect of disinfectant depends on concentration of the disinfectant, duration of disinfection and mycobacterial load in solution or on surface.
Avoid use of chlorine, benzalkonium chloride, cetylpyridinium chloride, quaternary ammonium compounds, and phenolic- or glutaraldehyde-based disinfectants as NTM are resistant to these chemicals.
Use of tap water or stored distilled water should be avoided.
Use of 70% alcohol and 5% phenol as disinfectant is recommended for bench surface cleaning and biosafety filters.
Autoclaving (at 131°C under 15 psi pressure) of plasticware and glassware used in laboratory is strongly recommended.
Laboratory workers should look for contamination by other micro-organism such as *Pseudomonas aeruginosa, Staphylococcus aureus, Nocardia, Aspergillus*, *etc*.
Incubation temperature for every species may vary between 27-45°C and requires constant monitoring.
Selective drug susceptibility testing should be done.
Laboratory workers should be aware about the patient's disease status and must co-ordinate the treating physician while reporting NTM species and subspecies.

BAL, bronchoalveolar lavage. *Source*: Ref. 130

These molecular tests though identify a limited number of NTM species, but fail to differentiate genetically closely related NTM species[133].

At present, DNA sequencing is the most accepted method for the identification and characterization of NTM species and subspecies[134,135]. These techniques include targeted gene sequencing and multi-locus sequence typing (MLST) that involve analysis of conserved genes such as *rpoB, hsp65,* 16S rRNA and 16S-23S rRNA internal transcribed spacer (ITS) region[134]. Targeted sequencing of single gene may identify a reasonable number of NTM species but sometimes may not distinguish species having close genetic association. MLST is preferred as multiple conserved genes are sequenced with this technique and on the basis of consensus analysis of different gene sequences, NTM species are identified more accurately[134].

Whole genome sequencing (WGS) is considered the gold standard for NTM species identification and is helpful in understanding the geographical and environmental distribution of NTM species. It is also useful to study healthcare-associated disease outbreaks and transmission[134]. WGS of NTM species can provide information on other characteristics such as virulence and resistance to various antimicrobial agents[135,136]. However, DNA sequencing is an expensive method and requires expertise[130]. This technique is not available in the routine laboratory set-up for NTM diagnosis in resource-limited countries[130].

Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS)-based

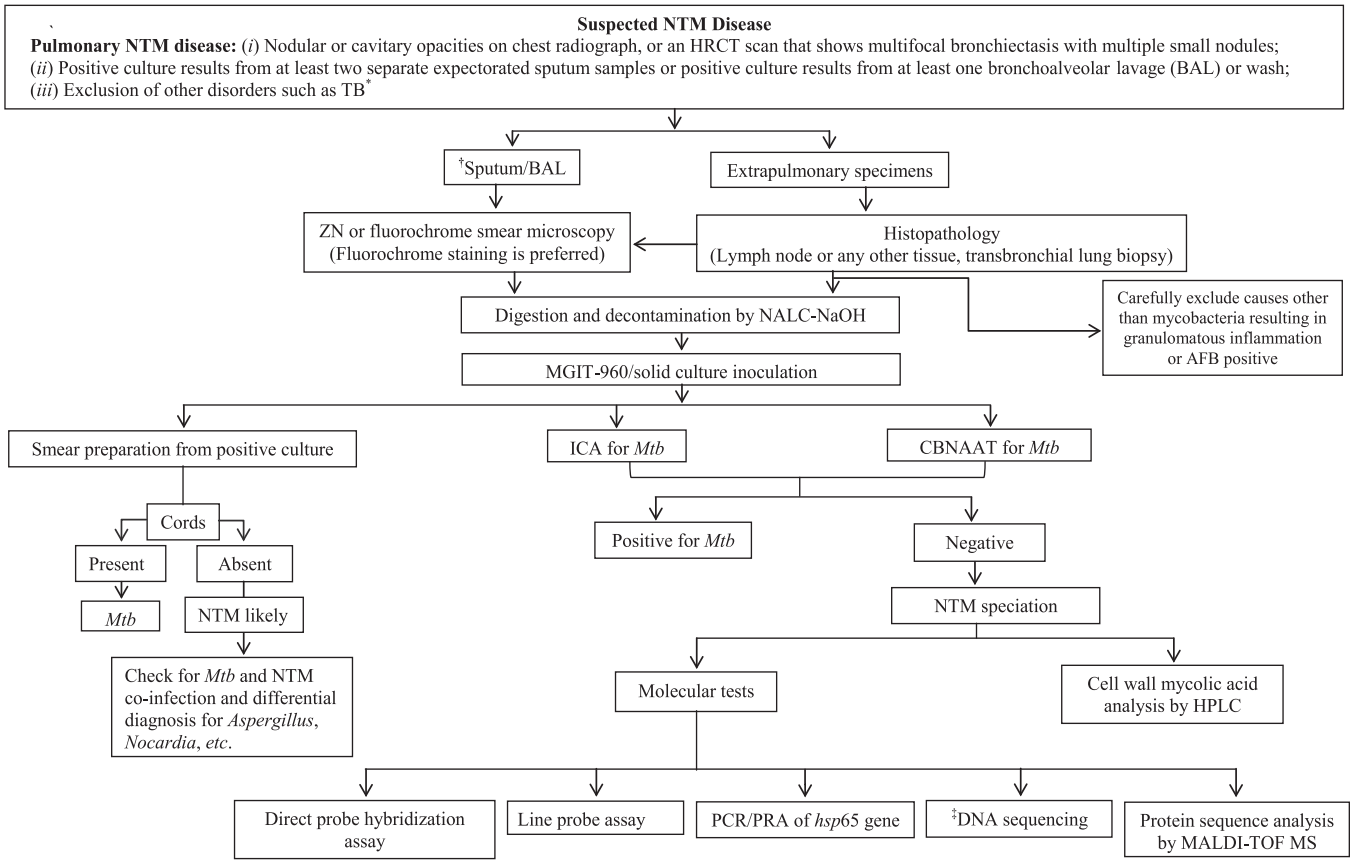

Fig. 8. Diagnostic algorithm for detection of NTM disease. *According to Ref. 16, consecutive three sputum samples are obtained, positive results from at least two separate expectorated sputum samples confirms the diagnosis. †While sputum collection, the patient should not rinse mouth with municipal or untreated water. Spontaneous sputum should be collected or sputum should be induced if no sputum is produced by patient. ‡Whole genome sequencing (NGS) and multi-locus targeted gene sequencing of gene such as 16S rRNA, *hsp*65, *rpoB,* 16S-23S rRNA internal transcribed region (ITS), *gyrB, dan*A, *rec*A and *sec*A. HRCT, high-resolution computed tomography; CSF, cerebrospinal fluid; ICA, immunochromatographic assay; CBNAAT, cartridge based nucleic acid amplification test; L-J, Lowenstein-Jensen media, HPLC: high-performance liquid chromatography, SGM, slowly growing mycobacteria; RGM, rapidly growing mycobacteria; DST, drug susceptibility testing; LPA, line probe assay; PNB: para-nitro benzoic acid; PCR/PRA, polymerase chain reaction/restriction endonuclease assay; MAC, *Mycobacterium avium* complex; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Source*: Refs 1, 17, 130.

analysis of conserved proteins is another technique available for NTM species identification[137]. MALDI-TOF-MS is considered the most rapid technique[137] which identifies around 160 NTM species[138]. However, like other techniques, MALDI-TOF-MS also fails to identify closely linked NTM species[130]. Details of various NTM identifications methods[130] are summarized in Table VIII.

Drug susceptibility testing (DST)

DST for NTM is controversial because of discrepancy between *in vitro* susceptibility and the treatment response[101]. DST should follow the Clinical and Laboratory Standards Institute (CLSI) guidelines[16]. CLSI recommends that phenotypic DST should be performed using broth microdilution method[18]. Both phenotypic and genotypic DST for MAC and *M. kansasii* are performed for initial and recurrent isolates. Acquired resistance for macrolide in MAC occurs due to point mutations in the 23S rRNA (*rrl*) gene and for amikacin due to mutations in 16S rRNA (*rrs*) gene (amikacin resistance is observed in MAC isolates cultured from sputum specimens of patients who were extensively exposed to the drug or related aminoglycosides)[18]. For MAC, DST against macrolides [clarithromycin is used as a class agent; minimum inhibitory concentration (MIC) cut-off: >32 µg/ml] and amikacin (MIC cut-off: >64 µg/ml for parenteral and >128 µg/ml for liposomal amikacin) and, for *M. kansasii*, DST against rifampicin (MIC >2 µg/ml) and clarithromycin are used (MIC ≥32 µg/ml)[128]. When *M. kansasii* is resistant against rifampicin, DST for amikacin, ciprofloxacin, doxycycline, linezolid, minocycline, moxifloxacin, rifabutin, and

Table VIII. Laboratory methods for non-tuberculous mycobacteria (NTM) identification

Method	Principle	Advantage(s)	Limitation(s)
Biochemical tests	Based on reaction products after niacin test, nitrate reduction, catalase activity, urease test, pyrazinamidase test, growth in the presence of p-nitrobenzoic acid, and hydrazide of thiophene 2-carboxylic acid	Low-cost tests and expert manpower not required	Time consuming and cumbersome tests; not useful for definitive species identification
HPLC	HPLC analysis of number of carbon atoms in mycolic acid found in the cell walls of NTM species	Cost of individual sample testing relatively inexpensive	Problematic for identification of rapidly-growing mycobacteria; limited ability to resolve some NTM groups/ complexes
PCR-RFLP	Analysis of the band patterns of restricted *hsp*65 gene fragments which are specific for different NTM species	Specialized equipment not required	Time-consuming; analysis restricted to a small fraction of the genome; requires trained staff; different sequences may share identical RFLP patterns thus it is not useful for definitive species identification especially with newer species/subspecies
Nucleic acid probes	Binding of ester-labelled gene DNA probes complementary to 16S rRNA gene	Provide quick results, as analysis may be performed directly on clinical samples	Identifies *M. avium, M. intracellulare, M. gordonae, M. kansasii* only; shows a cross-reactivity between MAC species and other NTM species
LPA	Reverse hybridization of genetic probes	Nucleic acid amplification increases sensitivity; low implementation costs	Useful for species identification but there can be cross reactivity with similar species
Gene sequencing	TGS Sequencing of single conserved gene MSLT: multiple conserved gene sequencing and consensus analysis for NTM species identification WGS	Useful for definitive species identification for most clinically relevant species; detects previously unknown mutations. Provides more accurate results than single TGS. Sequencing of entire genome allows detection of different genetic variants within the same population; helpful in understanding geographical and environmental distribution of NTM; useful in studying disease outbreaks and transmission of NTM; also provides information about other features such as virulence and resistance to various antimicrobial agents.	Specificity depends upon selection of gene target; closely related NTM species may not be identified; requires costly specialized equipment. Requires skilled manpower; sequence analysis dependent upon updated and accurate database. Expensive; data analysis is cumbersome and difficult; drug-resistant variants may be undetected if the drug susceptible variants are in majority; currently available sequencing platforms have problems with analysis of microsatellites.
MALDI-TOF MS	Analysis of conserved protein sequences	Identifies almost 160 NTM species; most rapid NTM identification test; may identify other organisms such as *Nocardia*, fungi, thus useful for differential diagnosis	High initial cost; cannot differentiate between subspecies of *M. abscessus* and species within the MAC, *M. fortuitum* and *M. mucogenicum* groups; limited database at present

HPLC, high-performance liquid chromatography; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism analysis; LPA, line probe assay; MALDI-TOF MS, matrix-assisted laser desorption time-of-flight mass spectrometry; rRNA, ribosomal RNA; TGS, targeted gene sequencing; MSLT, multi-locus sequence typing; WGS, whole genome sequencing; ITS, internal transcribed spacer; MAC, *Mycobacterium avium* complex. *Source*: Ref. 130

trimethoprim-sulfamethoxazole is recommended[18]. RGM species (and subspecies) show different drug resistance patterns[1], and DST should be selectively done for the following antibiotics: macrolides, amikacin, tobramycin, imipenem, trimethoprim-sulphamethoxazole, doxycycline, minocycline, tigecycline, cefoxitin and linezolid[1,65]. Information on an active *erm* (41) gene is important in RGM (*esp*. in *M. abscessus* subspecies) as it can lead to inducible resistance to macrolides[1,17]. In *M. abscessus* subsp *massiliense,* the *erm* (41) gene is nonfunctional owing to a large deletion, thus rendering the strains macrolide susceptible. The *erm* (41) gene is non-functional in some *M. abscessus* subsp. *abscessus* due to presence of C instead of T at the nucleotide 28 (arginine 10 instead of tryptophan 10)[18]. Constitutive resistance to macrolides can occur due to mutation in 23S rRNA gene[1]. Table IX describes various conditions of macrolide resistance among *M. abscessus* subspecies. *M. chelonae* is resistant to cefoxitin and sensitive to tobramycin[1].

Treatment of NTM disease

Principles of treatment

Several guidelines have been published for the management of NTM diseases[1,17-19,139]. While ATS/ IDSA deals with both pulmonary and extrapulmonary NTM diseases, the US Cystic Fibrosis Foundation and European Cystic Fibrosis Society (ECFS) guidelines, 2016[139], include consensus recommendations for the screening, investigation, diagnosis and management of NTM-PD in individuals with CF, and the BTS guidelines (2017)[1] and ATS/ ERS/ESCMID/IDSA guideline (2020)[18] deal with NTM-PDs. The treating physician should be well versed with the prevalence of various NTM species in the geographical area of his/her practice[1,17]. Despite repeated isolation of NTM, laboratory contamination and colonization in the host must be ruled out. As MAC is the most common cause of NTM-PD worldwide, causality association of repeated NTM isolation in the respiratory specimens should be carefully established after reviewing clinical and radiographic features[1,17]. Subsequently, the underlying predisposing structural lung disease should be identified and its severity should be evaluated. NTM-PD should be stratified into mild to moderate (non-severe) and severe NTM-PD (Box IV) on the basis of patient's systemic signs and symptoms, chest radiographic appearances and microbiologic features (acid-fast smear status, bacillary load, mycobacterial culture, NTM species and subspecies characterization)[1]. The conventional microbiological outcomes are smear status, culture conversion and relapse[1,140,141] (Box V).

The decision to start treatment should be made carefully as patients due to MAC remain stable without antibiotic treatment[1,17]. Early identification of certain clinical, radiographic and microbiological features that are associated with NTM-related progressive PD, is required. These include presence of severe symptoms, low body mass index (BMI) and poor nutritional status (*esp.* low albumin), lung cavitation, extensive disease, presence of comorbidity, elevated inflammatory markers, and positive AFB smears and isolation of more virulent NTM species[18,94,142,143]. Recent ATS/ERS/ ESCMID/IDSA guideline (2020)[18] suggests initiation of treatment rather than watchful waiting, especially in the context of positive AFB sputum smears and/or cavitary lung disease. Whereas, a watchful waiting is preferred in patients with mild signs and symptoms of disease, higher chances of drug intolerance and adverse drug reactions and NTM species less responsive to treatment (*e.g., M. abscessus*). In such cases, treatment should be initiated after counselling the patient about potential adverse effects of antimicrobial therapy, the uncertainties surrounding the benefits of antimicrobial therapy, and the possibility for recurrence including reinfection (specifically in nodular-bronchiectatic disease setting). It is also recommended that treatment

Table IX. Interpretation of extended clarithromycin susceptibility results for *Mycobacterium abscessus*

Clarithromycin susceptibility (days 3-5)	Clarithromycin susceptibility (day 14)	Genetic implication	*M. abscessus* subspecies	Macrolide susceptibility phenotype
Susceptible	Susceptible	Dysfunctional *erm* (41) gene	*M. abscessus. massiliense*	Macrolide susceptible
Susceptible	Resistant	Functional *erm* (41) gene	*M. abscessus. abscessus* *M. abscessus. bolletii*	Inducible macrolide resistance
Resistant	Resistant	23S ribosomal RNA point mutation	Any	High-level constitutive macrolide resistance

Source: Reproduced with permission from Ref. 1

regimens should be designed by experts in the management of complicated NTM infections[18].

NTM-PD is generally treated with a drug regimen, consisting of 3-4 antibiotics, administered either daily or thrice weekly depending on the severity of disease, patient's tolerance of drugs and occurrence of side effects, and the therapy is continued for at least 12 months following sputum conversion[17,18]. Table X summarizes the treatment durations of pulmonary and extrapulmonary NTM diseases[144] due to different species.

A significant proportion of patients with NTM-PD discontinues the prescribed treatment because of lengthy duration and occurrence of side effects[145]. The treatment regimens vary depending on the isolation of NTM species, clinical phenotypes and drug susceptibility profiles, leading to varying therapeutic responses. The variable treatment responses are related to several factors such as NTM species (*M. avium* vs. *M. abscessus*) and subspecies (*M. abscessus* subsp. *massiliense* vs. *M. abscessus* subsp. *abscessus*), disease phenotype [fibrocavitary vs. nodular bronchiectatic (NB)] and the treatment regimen (drug treatment regimen with macrolide vs. without macrolide)[146-148].

Box IV. Definitions of mild-moderate and severe non-tuberculous mycobacteria (NTM) disease

Mild-moderate (non-severe disease)
Mild-moderate symptoms
No signs of systemic illness
Absence of lung cavitation and extensive lung disease
AFB smear-negative in the pulmonary specimens
Severe disease
Presence of severe symptoms and signs of systemic illness
Presence of lung cavitation and extensive lung involvement
Pulmonary specimens positive for AFB smear

AFB, acid-fast bacilli; NTM-PD, non-tuberculous mycobacterial pulmonary disease. *Source*: Ref. 1

NTM-PD due to MAC is treated with a drug regimen comprising rifampicin (or rifabutin in HIV-positive individuals to avoid drug-drug interactions[19]), ethambutol and macrolide (azithromycin or clarithromycin; some patients tolerate azithromycin better)[1,17]. There is an *in vitro* synergy of antimycobacterial action between rifampicin and ethambutol as the latter destabilizes mycobacterial cell wall and facilitates rifampicin entry into the Mycobacteria to its target site, the RNA polymerase[149,150]**.** These two drugs also prevent development of macrolide resistance[151]. Neither isoniazid nor moxifloxacin is much active against MAC; clofazimine and amikacin are good alternatives. The BTS guidelines (2017)[1] and ATS/ERS/ESCMID/IDSA guideline (2020)[18] recommend intermittent three times-weekly treatment for non-cavitary (non-severe) MAC-PD due to potential benefits, better treatment adherence and comparable efficacy[1,152]. As per guidelines, intravenous or nebulized amikacin can be added as the fourth drug for the initial three months in patients with severe or macrolide-resistant MAC-PD[1] (Table XI). The pooled treatment success rates in MAC-PD in the five systematic reviews ranged from 32 to 65 per cent, and 12 to 16 per cent of the enrolled patients had not completed treatment[153-157].

Miwa *et al*[158] in a preliminary open-label study compared three-drug regimen (clarithromycin, ethambutol and rifampicin) with two-drug regimen (clarithromycin and ethambutol) and demonstrated the rate of sputum culture conversion at 40.6 per cent with three-drug regimen versus 55 per cent with two-drug regimen, suggesting that two-drug regimen was not inferior to three-drug regimen. Further, the incidence of adverse events

Box V. Definitions for microbiological outcomes in non-tuberculous mycobacterial (NTM) disease

Culture conversion: Three consecutive negative mycobacterial sputum cultures collected over a minimum of three 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 six months of NTM treatment

*Jhun *et al*[140] defined refractory NTM-PD as persistent positive sputum cultures after at least 6 months of multidrug treatment instead of 12 month GBT. In addition, administration of ARIKAYCE plus GBT in patients with MAC pulmonary disease resulted sputum culture conversion by month 6 in 29% cases in comparison to 9% who were on GBT alone. GBT, guideline based treatment. *Source*: Ref. 1

Table X. Durations of treatment for different non-tuberculous mycobacteria (NTM) diseases

Site of NTM infection	Treatment duration/adjunct therapies
Pulmonary	Twelve months after sputum culture becomes negative.
Disseminated disease*	Twelve months after blood culture becomes negative. Secondary prophylaxis is required after this till CD4 count is >100 cells/µl for three months.
Lymphadenitis†	Surgery alone may be curative in children with NTM cervical lymphadenitis (*i.e.*, MAC). Combination drug therapy is recommended when surgical debridement is not complete or in the setting of disseminated disease in an immunocompromised host. Duration of treatment is variable. In patients with single peripheral lymph node, surgical excision is the treatment of choice. In patients with disseminated disease, treatment duration is longer.
Skin and soft tissue	Four to six months of combination therapy and adjunctive surgery may be done.
Vertebral disease	Twelve months of drug treatment preferred and adjunctive surgery may be done.
Other bone disease	Six to nine months of drug therapy and adjunctive surgery may be done.
Catheter-associated bloodstream infection	Remove iv catheter, if possible. Treatment should be given 1-3 months depending on the immune status of the individual and NTM species.

*Disseminated disease: Involvement of two or more organs through hematogenous spread. Lung involvement may or may not be present and pulmonary involvement occurs in 2.5-8% of patients with disseminated MAC disease in advanced HIV/AIDS. †In high TB burden countries, *Mtb* is the commonest cause of lymphadenitis. iv, intravenous. *Source*: Reproduced with permission from Ref. 144

Table XI. Suggested antibiotic regimens for adults with *Mycobacterium avium* complex (MAC)-pulmonary disease

MAC-pulmonary disease	Antibiotic regimen
Non-severe MAC-pulmonary disease (*i.e.*, AFB smear-negative respiratory tract samples, no radiological evidence of lung cavitation or severe infection, mild-moderate symptoms, no signs of systemic illness)	Rifampicin 600 mg 3× per week and ethambutol 25 mg/kg 3× per week and Azithromycin 500 mg 3× per week or clarithromycin 1 g in two divided doses 3× per week antibiotic treatment should continue for a minimum of 12 months after culture conversion.
Severe MAC-pulmonary disease (*i.e.*, AFB smear-positive respiratory tract samples, radiological evidence of lung cavitation/severe infection, or severe symptoms/signs of systemic illness)	Rifampicin 600 mg daily and ethambutol 15 mg/kg daily and azithromycin 250 mg daily or clarithromycin 500 mg twice daily and consider intravenous amikacin for up to three months or nebulized amikacin antibiotic treatment should continue for a minimum of 12 months after culture conversion.
Clarithromycin-resistant MAC-pulmonary disease	Rifampicin 600 mg daily and ethambutol 15 mg/kg daily and isoniazid 300 mg (+pyridoxine 10 mg) daily or moxifloxacin 400 mg daily and consider intravenous amikacin for up to three months or nebulized amikacin antibiotic treatment should continue for a minimum of 12 months after culture conversion.

AFB, acid-fast bacilli. *Source*: Reproduced with permission from Ref. 1

leading to treatment discontinuation was higher with the three-drug regimen (37.2 vs. 26.6%)[158] .

Koh *et al*[159] evaluated 481 treatment-naïve patients with MAC lung disease who underwent antibiotic treatment for ≥12 months between January 2002 and December 2013. Nearly 58 per cent had non-cavitary NB disease, 17 per cent had cavitary NB disease and 25 per cent had fibrocavitary disease. The treatment outcomes and redevelopment of NTM lung disease after treatment completion differed by the clinical phenotype of MAC lung disease. Cavitary disease was independently associated with unfavourable outcomes. The NB form was an independent risk factor for the redevelopment of NTM lung disease. Of the 29 per cent of favourable outcomes, redevelopment of NTM lung disease occurred with the same MAC species in 55 per cent patients. In patients with recurrent MAC lung disease due to the same species, genotyping revealed that 74 per cent of cases were attributable to reinfection and 26 per cent to relapse[159].

Addition of once-daily administration of amikacin liposome inhalation suspension (ALIS) (supplied in single-use vials delivering 590 mg amikacin to the nebulizer), also known as ‘Arikayce’ to standard guideline-based therapy (GBT) in adults with refractory MAC lung disease (with amikacin-susceptible MAC lung disease and MAC-positive sputum cultures despite at least six months of stable therapy considered

to be macrolide-based multidrug treatment), has been reported[141]. Addition of ALIS to GBT for the treatment of refractory MAC lung disease achieved significantly greater culture conversion by six month than GBT alone. Respiratory adverse events (primarily dysphonia, cough and dyspnoea) were reported more (87.4%) in patients receiving ALIS+GBT than those receiving GBT alone (50%)[141]. Patients with limited and refractory MAC-PD should be considered for lung resection[134].

Patients with clarithromycin-resistant MAC-PD should be treated with rifampicin, ethambutol and isoniazid or a quinolone and intravenous amikacin or nebulized amikacin (if intravenous amikacin is not tolerated or impractical to administer or is contraindicated) for initial three months[1] (Table XI). The treatment of macrolide-resistant (MR) MAC-PD is challenging because of poor sputum culture conversion rates (15-36%) and high mortality rates at two year (9-15%) and five-year (47%)[160,161]. A recent systematic review and meta-analysis of nine studies reported poor treatment outcomes in MR-MAC-PD with overall 21 per cent sputum culture conversion rate and 10 per cent one-year all-cause mortality with no difference between NB and FC types of MR-MAC-PD[162]. Despite the combination of multiple antibiotics including ALIS and surgical resection, the treatment outcomes of MR-MAC-PD remained poor.

Patients with NTM-PD due to rifampicin-sensitive *M. kansasii* are treated with a treatment regimen similar to pulmonary TB[1,17] comprising rifampicin, ethambutol and isoniazid along with pyridoxine for a fixed duration of 12 months instead of 12 months beyond culture conversion[18] . Even one-time isolation of *M. kansasii* from patient's sputum sample is considered pathogenic and should be treated immediately[18] (Table XII). Because MICs (minimum inhibitory concentrations) of isoniazid are higher as compared to *Mtb*, therefore, macrolide (clarithromycin or azithromycin) is preferred over isoniazid for the treatment of *M. kansasii*[163]. Pyrazinamide is not recommended for *M. kansasii* pulmonary disease as the organism is naturally resistant to pyrazinamide (a prodrug) due to reduced pyrazinamidase activity preventing conversion of the drug into pyrazinoic acid which is an active bactericidal compound[164]. Cure rates for rifampicin-sensitive *M. kansasii* have been >98 per cent[17]. Table XII describes treatment regimens for rifampicin-sensitive and rifampicin-resistant *M. kansasii*[1].

Table XIII details the treatment of PD due to *M. xenopi*. While four-drug regimen (rifampicin, ethambutol, macrolide and moxifloxacin) is used to treat non-severe disease, intravenous amikacin or nebulized amikacin is added to the regimen as a fifth drug for severe disease[1]. In a retrospective matched cohort study comparing *M. xenopi* PD to MAC-PD, 24-month mortality was higher in *M. xenopi*-PD with comorbidities, especially COPD. Rifampicin was less frequently used in *M. xenopi*[165].

Treatment response of macrolide-containing regimen in patients with *M. malmoense* NTM-PD is better than that of MAC or *M. xenopi*[90]. Table XIV provides the details of drug regimen. Treatment for other slowly growing NTM can be extrapolated from common NTM species. Isolation of *M. simiae* is rarely associated with true infection. Limited success is seen in *M. simiae* infection with rifampicin- and ethambutol-based drug regimen[166], and a combination of amikacin and clofazimine may be used to construct a drug regimen to treat the infection[167].

The treatment details of PD due to *M. abscessus* are provided in Table XV, and antibiotic combination is administered according to the DST profile. In patients with *M. abscessus,* pulmonary disease is caused

Table XII. Suggested antibiotic regimen for adults with *Mycobacterium kansasii*-pulmonary disease

M. kansasii-pulmonary disease	Antibiotic regimen
Rifampicin-sensitive *M. kansasii*-pulmonary disease*	Rifampicin 600 mg daily and ethambutol 15 mg/kg daily and isoniazid 300 mg (with pyridoxine 10 mg) daily; or azithromycin 250 mg daily or clarithromycin 500 mg twice daily. Antibiotic treatment should continue for a minimum of 12 months after culture conversion.
Rifampicin-resistant *M. kansasii*-pulmonary disease*	Azithromycin 250 mg once daily or clarithromycin 500 mg twice daily and ethambutol 15 mg/kg daily and moxifloxacin 400 mg once daily; or isoniazid 300 mg once daily (with pyridoxine 10 mg) and ethambutol 15 mg/kg daily and moxifloxacin 400 mg once daily.

*DST guided three-drug regimen from above mentioned antibiotic agents. Pyrazinamide is not recommended for *M. kansasii* pulmonary disease as the organism is naturally resistant to pyrazinamide (a prodrug) due to reduced pyrazinamidase activity preventing conversion of the drug into pyrazinoic acid which is an active bactericidal compound. *Source*: Adapted with permission from Refs 1, 18

Table XIII. Suggested antibiotic regimens for adults with *Mycobacterium xenopi*-pulmonary disease

M. xenopi-pulmonary disease	Antibiotic regimen
Non-severe *M. xenopi*-pulmonary disease (*i.e.*, AFB smear-negative respiratory tract samples, no radiological evidence of lung cavitation or severe infection, mild-moderate symptoms, no signs of systemic illness)	Rifampicin 600 mg daily and ethambutol 15 mg/kg daily and azithromycin 250 mg/daily or clarithromycin 500 mg twice daily and moxifloxacin 400 mg daily or isoniazid 300 mg (+pyridoxine 10 mg) daily. Antibiotic treatment should continue for a minimum of 12 months after culture conversion.
Severe *M. xenopi*-pulmonary disease (*i.e.*, AFB smear-positive respiratory tract samples, radiological evidence or lung cavitation/severe infection, or severe symptoms/signs of systemic illness)	Rifampicin 600 mg daily and ethambutol 15 mg/kg daily and azithromycin 250 mg/daily or clarithromycin 500 mg twice daily. Moxifloxacin 400 mg daily or isoniazid 300 mg (+pyridoxine 10 mg) daily and consider intravenous amikacin for up to 3 months or nebulized amikacin. Antibiotic treatment should continue for a minimum of 12 months after culture conversion.

Source: Reproduced with permission from Ref. 1

Table XIV. Suggested antibiotic-regimens for adults with *Mycobacterium malmoense*-pulmonary disease

M. malmoense-pulmonary disease	Antibiotic regimens
Non-severe *M. malmoense*-pulmonary disease (*i.e.*, AFB smear-negative respiratory tract samples, no radiological evidence of lung cavitation or severe infection, mild-moderate symptoms, no signs of systemic illness)	Rifampicin 600 mg daily and ethambutol 15 mg/kg daily and azithromycin 250 mg/daily or clarithromycin 500 mg twice daily. Antibiotic treatment should continue for a minimum of 12 months after culture conversion.
Severe *M. malmoense*-pulmonary disease (*i.e.*, AFB smear-positive respiratory tract sample, radiological evidence of lung cavitation/severe infection or sever symptoms/signs of systemic illness)	Rifampicin 600 mg daily and ethambutol 15 mg/kg daily and azithromycin 250 mg/daily or clarithromycin 500 mg twice daily and consider intravenous amikacin for up to 3 months or nebulised amikacin. Antibiotic treatment should continue for a minimum of 12 months after culture conversion.

Source: Reproduced with permission from Ref. 1

Table XV. Suggested antibiotic regimens for adults with *Mycobacterium abscessus*-pulmonary disease

M. abscessus	Antibiotic regimen
Clarithromycin sensitive isolates	Initial phase: ≥1 month[†] Intravenous amikacin 15 mg/kg daily or 3×per week[‡] and intravenous tigecycline 50 mg twice daily and where tolerated intravenous imipenem 1 g twice daily and where tolerated oral clarithromycin 500 mg twice daily or oral azithromycin 250-500 mg daily. Continuation phase: Nebulized amikacin[‡] and oral clarithromycin 500 mg twice daily or azithromycin 250-500 mg daily and 1-3 of the following antibiotics guided by drug susceptibility results and patient tolerance: Oral clofazimine 50-100 mg daily[§] Oral linezolid 600 mg daily or twice daily Oral moxifloxacin 400 mg daily
Inducible macrolide-resistant isolates or constitutive macrolide-resistant isolates	Initial phase: ≥1 month[†] Intravenous amikacin 15 mg/kg daily or 3× per week[‡] and intravenous tigecycline 50 mg twice daily and where tolerated intravenous imipenem 1 g twice daily. Continuation phase: Nebulized amikacin[‡] and 2-3 of the following antibiotics guided by drug susceptibility results and patient's tolerance: Oral clofazimine 50-100 mg daily[§] Oral linezolid 600 mg daily or twice daily Oral moxifloxacin 400 mg daily

[†]Due to the poor response rates in patients with inducible or constitutive macrolide-resistant isolates and the greater efficacy of antibiotics administered through the intravenous route, extending the duration of intravenous antibiotic therapy to 3-6 months in those who can tolerate it may be the most appropriate treatment strategy in this subgroup of patients. [‡]Substitute intravenous/nebulized amikacin with an alternative antibiotic if the *M. abscessus* is resistant to amikacin (*i.e.*, MIC >64 mg/l or known to have a 16S rRNA gene mutation conferring constitutive amikacin resistance). [§]Start clofazimine during the initial phase of treatment if tolerated as steady-state serum concentrations may not be reached until ≥30 days of treatment. Lower dose of intravenous tigecycline (25-50 mg once daily) may be given if not tolerated. *Source*: Adapted with permission from Ref. 1

by strains with inducible and mutational macrolide resistance, a macrolide-based regimen is recommended if the drug is used as an immunomodulator (macrolide is not considered as active drug in multidrug regimen)[18]. A precise identification of subspecies along with information on *erm* (41) gene is important in *M. abscessus* infection[1] because of a variable treatment response. The treatment outcomes among the three subspecies of *M. abscessus* differ due to *erm* (41) gene and inducible and constitutive resistance to macrolides[1] (Table IX). About 15 per cent of *M. abscessus* strains have a T to C mutation at position 28 in *erm* (41) gene, making them macrolide susceptible[168].

A systematic review and meta-analysis of the studies on the effect of chemotherapy on pulmonary *M. abscessus* with macrolide-containing regimens reported adverse microbiological outcomes with frequent recurrences according to the subspecies[169]. A good outcome was defined as sustained sputum culture conversion (SSCC) without relapse. Macrolide-containing regimens achieved SSCC in only 34 per cent (77/233) patients with new *M. abscessus* subsp. *abscessus* vs. 54 per cent (117/141) in those with *M. abscessus* subsp. *massiliense*. In refractory disease, SSCC was achieved in 20 per cent of patients, which was not significantly different across subspecies. The proportion of patients with good outcomes (SSCC rate without relapse) was 23 per cent (52/223) with *M. abscessus* subsp. *abscessus* versus 84 per cent (118/141) with *M. abscessus* subsp. *massiliense* disease. The pooled sputum culture conversion rate was 20 per cent (95% confidence interval, 7-36%), which on follow up after stopping therapy for 12 months was not significantly different across the mycobacterial species. Overall, disease recurrence in *M. abscessus* subsp. *abscessus*-infected patients was 40 per cent versus seven per cent in *M. abscessus* subsp. *massiliense*-infected patients. The odds ratio of recurrence in *M. abscessus* subsp. *abscessus*-infected versus *M. abscessus* subsp. *massiliense*-infected patients was 6.2[169].

In patients with lung infection due to *M. fortuitum,* the underlying GERD should be carefully evaluated and treated[17,170]. Surgical excision is the treatment of choice for younger children with cervicofacial lymphadenitis due to NTM[105,171]. Treatment of the skin disease due to *M. marinum* depends on the extent of lesions, hence drug regimen comprising rifampicin and ethambutol or ethambutol and clarithromycin is administered for a single small lesion, whereas triple-drug regimen of rifampicin, ethambutol and a macrolide is used for severe disease[172-174]. Adjunctive surgical debridement is recommended for the underlying bone and joint involvement. Eight-week drug regimens of rifampicin with either clarithromycin or quinolone are administered for the treatment of *M. ulcerans* skin disease[175,176]. Disseminated skin and subcutaneous abscesses caused by RGM can be treated with two-drug regimen based on DST results for four months[17] in addition to surgical debridement[177,178]. For *M. fortuitum* infection, drug regimen may include a combination of co-trimoxazole, tobramycin, imipenem, doxycycline and fluoroquinolones[17]; *M. chelonae* infection is treated with two-drug combinations of tobramycin, linezolid, macrolides and imipenem[17,177,178]. *M. abscessus* infections may be treated with a combination of the following antibiotics: amikacin, linezolid, cefoxitin, macrolides and imipenem based on DST results[17,18]. The utility of macrolides depends on *erm* (41) gene functional status (Table IX).

Recent recommendations for treating disseminated MAC disease in HIV/AIDS patients are provided in Box VI[19]. Non-steroidal anti-inflammatory drugs (NSAIDS) may be used in HIV patients experiencing moderate-to-severe symptoms of immune reconstitution inflammatory syndrome (IRIS), and short-term course of corticosteroids for 4-8 wk can be used if symptoms persist.

Inhaled antibiotics for NTM-PD

Similar to TB treatment, drug treatment regimens comprising 3-4 drugs are used for treating NTM-PD for longer periods with high discontinuation rates (9-39%) due to significant side effects[145,154,179,180]. Use of inhaled drugs has demonstrated successful treatment outcomes in bronchial asthma, COPD and *Pseudomonas aeruginosa* infections in CF patients while achieving higher drug concentrations at the disease site without developing significant systemic side effects at the same time[155]. Similar approach can be considered in NTM-PD to deliver higher drug concentrations to the infected lungs with minimal extrapulmonary exposure to avoid adverse events. Inhaled amikacin along with other oral drugs is already used in patients with severe NTM-PD[1,180-182]. Development of inhaled clofazimine suspension for administration via nebulizer device in NTM-PD treatment is in progress[180]. In addition, studies using inhaled recombinant granulocyte-macrophage

Box VI. Measures for preventing non-tuberculous mycobacteria (NTM)
Measures to reduce health care-and hygiene-associated NTM disease
Avoid the following
Exposure of injection sites, intravenous catheters and surgical wounds to tap water and tap water-derived fluids
Cleaning of endoscopes with tap water
Contamination of clinical specimens with tap water and ice
Use of benzalkonium chloride as a skin disinfectant prior to local injections
Household and personal measures
Avoid using saunas, hot tubs or any water with an aerator. Hot water usage should be done in proper ventilation
Replacement of shower heads at regular intervals; temperature of water heater should be ≥54.4°C
Sterilized water should be used in humidifiers; avoid ultrasonic humidifiers
Take steps to reduce GERD; avoid foods that may trigger it and avoid vulnerable body positions that may cause aspiration
NTM-associated hypersensitivity lung disease
Ensure regular cleaning of indoor pools, hot tubs and hot water pipes
GERD, gastroesophageal reflux disease. *Source*: Ref. 10

colony-stimulating factor and exogenous nitric oxide gas are in progress to evaluate their antibacterial effect on *M. abscessus*[183].

Non-pharmacologic treatment of pulmonary NTM disease

In addition to pharmacological therapy, other non-pharmacological measures can be tried for treating the underlying lung disease[184] These include techniques for mucus clearance such as nebulization using hypertonic saline, aerobic exercises, chest physiotherapy, postural drainage, use of oscillating positive expiratory pressure devices and high-frequency chest wall oscillation. Intake of balanced diet containing adequate calories and proteins to maintain ideal body weight is essential in the management of NTM diseases[185]. Following recovery, patients should avoid exposure to minimize re-infection from environmental sources such as hot tubs, use of tap water in humidifiers and continuous positive airway pressure units, use of specialized filtration systems in household plumbing and exposure to soil and dust.

Surgical intervention

Surgery may be considered in carefully selected individuals with NTM-PD. These patients should have localized structural lung disease and good pulmonary functions without having impaired gas exchange[1,17,162]. The role of a pulmonary and/or infectious disease specialist, a respiratory therapist and a nutrition expert is crucial for a successful surgical outcome[186]. A review of retrospective anatomic lung resection for NTM-PD in 236 consecutive patients revealed minimal mortality and morbidity and reported that 80 per cent of patients had MAC-PD and had received DST-guided antibiotic treatment prior to surgery[187]. Data from the annual survey between 2008 and 2012 by the Japanese Association for Thoracic Surgery (JATS) have demonstrated a steady increase in the number of NTM surgeries[188]. In patients with extrapulmonary NTM disease, surgical intervention may be required through aggressive debridement or removal of implanted material[189]. Surgical excision is the treatment of choice in patients with solitary peripheral lymph node involvement due to NTM, especially in children[105,106,189].

Monitoring of drug toxicities

Drugs used for the treatment of NTM diseases are associated with several adverse events especially in elderly individuals and HIV/AIDS patients with multisystem involvement. During follow up, patients should be carefully monitored for side effects[1,144,190]. Table XVI provides the details of adverse events and laboratory monitoring.

Prevention

Box VI provides details of various preventive measures to reduce NTM disease in different settings, especially those due to contamination of disinfectants, ice, wounds, injection sites, catheters, endoscopes, *etc*., can be prevented by proper sterilization[2,3]. Avoiding the use of tap water is considered a key step to prevent NTM infections in the hospital settings. Further, patients undergoing cardiac surgery and transplants should receive extra attention[10]. Besides different drug regimens, certain non-pharmacological options are

Table XVI. Drugs used in non-tuberculous mycobacteria (NTM) disease, monitoring and adverse drug reactions

Drug	Dosing	Monitoring	Serious adverse effects
Clarithromycin (oral) or iv infusion (500 mg twice daily through a large proximal vein if not tolerated orally)	500 mg twice daily or 500 mg PO twice daily TIW	Monitor QTc prolongation if administered with drugs having potential to prolong QTc, audiograms at baseline, one month, and then every three months; inhibits hepatic metabolism of several agents including rifabutin and some protease inhibitors. Drug levels need not be monitored. Avoid concomitant use of ivabradine, ticagrelor, decrease dose of rifabutin if co-administered with clarithromycin. Increases plasma concentrations of antileptics, phenytoin, carbamazepine (monitor plasma levels), ciclosporin, linezolid (monitor drug level), sirolimus and tacrolimus; coumarins: warfarin; theophylline	GI disturbances including taste perversion, headache, QTc prolongation especially when co-administered with drugs that have the potential to prolong the QT interval, ototoxicity, dermatological: (toxic epidermal necrolysis and Stevens-Johnson syndrome) hepatic dysfunction, *Clostridium difficile*-induced diarrhoea.
Azithromycin (oral)	250-500 mg daily	Monitor QTc prolongation if administered with other drugs having potential to prolong QTc; audiogram at baseline, one month, and then every three months	GI disturbances, QTc prolongation when administered with drugs having potential to increase QTc, ototoxicity, hepatitis
Ethambutol (oral)	15 mg/kg per day or 25-30 mg/kg thrice weekly. Target level 2-6 mg/l; drug levels routinely not measured; only in special situations like renal impairment and poor treatment response.	Crcl ≥30 ml/min: no dose adjustment; Crcl <30 ml/min: 15-25 mg thrice weekly; baseline eye examination and monthly visual acuity tests/ colour discrimination tests (Ishihara). Baseline and every three months. Funduscopic monitoring.	Dose dependent optic (retrobulbar) neuropathy (>30 mg/kg/day or 15-25 mg/kg in CKD); generally, reverses on prompt discontinuation; red-green colour blindness; risk increases with concurrent use of isoniazid; hyperuricemia. Rare: interstitial nephritis, cholestatic jaundice, neutropenia and thrombocytopenia, reversible cutaneous hypersensitivity disappearing on desensitisation
Rifampicin (oral)	<50 kg: 450 mg once daily or >50 kg: 600 mg once daily (should be taken 30-60 min before food or 2 h after food)	Monitor LFTs, including ALT, AST, alkaline phosphatase, and bilirubin levels	Red/orange discoloration of secretions, GI disturbances, hepatitis, hypersensitivity (fever, rash)
Rifabutin (oral)	Routinely 300 mg daily, rarely 450 mg; may administer thrice weekly	Monitor LFTs, including ALT, AST, alkaline phosphatase, and bilirubin levels	Red/orange discolouration of secretions; GI disturbances, loss of taste, hypersensitivity, polyarthralgia, polymyalgia, anterior uveitis and leukopenia (in combination with clarithromycin)

Contd...

Drug	Dosing	Monitoring	Serious adverse effects
Isoniazid (oral)	5 mg/kg per day (maximum of 300 mg)	Monitor LFTs including ALT and AST levels in patients at risk	Hypersensitivity reaction, hepatitis, peripheral neuropathy, haematological abnormalities (agranulocytosis, megaloblastic anaemia, thrombocytopenia), psychosis (rare) drug induced lupus (rare), arthralgia, rhabdomyolysis
Amikacin (intravenous)	15 mg/kg once daily for 5 days (Monday-Friday) or 15-25 mg thrice weekly. Consider starting with 8-10 mg/kg per day for the elderly and patient with mild renal impairment and titrate upward to goal C_{max}.	Target C_{max} 25-35 µg/ml for daily dose and >35-45 µg/ml with thrice weekly administration. Audiometry should be done at baseline and subsequently monthly. A final audiometry should be done 2 months after the final dose. Monitor renal functions weekly in first month, twice weekly in second month and fortnightly thereafter. Preferably avoid or dose adjustment required in CKD.	Nephrotoxicity: Higher chances in old age and with prolonged use. Ototoxicity: auditory>vestibular; ototoxicity includes hearing loss, loss of balance and tinnitus. Hearing loss occurs first and is detected by audiometric testing. Ototoxicity in audiogram is defined as 20 dB loss from baseline at any one test frequency or a 10 dB loss at any two adjacent test frequencies. Hearing loss is usually permanent. Vertigo, loss of balance and tinnitus.
Amikacin (inhalation) Arikayce (liposome inhalation)	250 mg/ml solution diluted with 3 ml of 0.9% sodium chloride daily, can be increased to 500 mg once daily depending on patient's tolerance. In patient with reactive airways disease, inhaled bronchodilators can be administered prior to administration to reduce the risk of wheezing and coughing. Oral inhalation, used in a limited and specific population of patients. Use Arikayce vials only with Lamira Nebulizer system. The recommended dosage in adults is once daily oral inhalation of the contents of one 590 mg/8.4 ml of Arikayce vial. Pre-treatment with inhaled bronchodilator should be considered in patients with a history of hyperactive airway disease.	Observe amikacin trough and creatinine levels after 1-2 wk of therapy, then repeat in one month; audiogram at baseline and then in one month; if all normal, then creatinine and amikacin trough levels, and audiograms every three months. Arikayce use should be reserved for those adults who have limited or no alternative treatment options, for the treatment of MAC lung disease as part of a combination antibacterial drug regimen. This indication is approved under accelerated approval based on achieving sputum culture conversion (defined as 3 consecutive negative monthly sputum cultures) by month 6. Arikayce has only been studied in refractory MAC lung disease (patient who did not achieve negative sputum cultures after minimum of 6 consecutive mo of multidrug background regimen therapy)	Dysphonia, respiratory concerns (bronchiectasis exacerbation, dyspnoea); watch for systemic adverse effects as well. Arikayce related increased risk of respiratory adverse events include, common: dysphonia (50%) and coughing (30%), and uncommon: hypersensitivity pneumonitis, haemoptysis, bronchospasm, exacerbation of underlying pulmonary disease. Other adverse reactions include ototoxicity, nephrotoxicity, neuromuscular blockade and embryo-foetal toxicity when administered to a pregnant woman.

Contd...

Drug	Dosing	Monitoring	Serious adverse effects
Linezolid (oral or intravenous)	600 mg daily; may decrease dose to 300 mg after 3-6 months	Careful monitoring for haematological toxicity, lactic acidosis, peripheral and optic neuropathy (often reversible); pyridoxine 100 mg can be administered to prevent haematological toxicity; to prevent serotonin syndrome, avoid tyramine rich food items and medications known to raise serotoin production; monitor CBC count with differential count weekly for 2 wk, then twice weekly.	Haematological toxicity, lactic acidosis, myelosuppression, peripheral and optic neuropathy and serotonin syndrome. Haematological toxicity (early) and lactic acidosis may occur in a few weeks to months whereas neurological toxicity occurs after 3-4 months (late)
Levofloxacin (oral)	500-1000 mg daily	Consider ECG monitoring if additional risk factors present; Dose adjustment required in CKD. Crcl ml/min=750-1000 mg daily, Crcl <30 ml/min=750-1000 mg thrice weekly	GI upset, dizziness, hypersensitivity, photosensitivity, headache, insomnia, tendinitis, tendon rupture, peripheral neuropathy, CNS effects, headache, agitation, depression, paranoia, seizures, QTc prolongation on ECG
Moxifloxacin (oral)	400 mg daily	Consider ECG monitoring if additional risk factors present; no dose adjustment is required in CKD; hepatobiliary excretion; avoid concomitant use of antacids with aluminium sucralfate, phosphate binders, calcium, iron, or aluminium containing medications to avoid malabsorption	Tendinitis, tendon rupture, peripheral neuropathy, CNS effects, QTc prolongation on ECG
Doxycycline (oral)	100 mg twice daily	Monitor clinical symptoms of the patient	GI disturbances, photosensitivity
Minocycline (oral)	100 mg twice daily	Monitor clinical symptoms of the patient	GI disturbances, photosensitivity, hyperpigmentation of the skin and CNS effects
Trimethoprim/ sulphamethoxazole (oral)	One double-strength twice or thrice daily	Monitor potassium at baseline, 2 wk, 12 wk then monthly	GI disturbances, cytopenia, renal failure, hyperkalemia
Bedaquiline (oral)	400 mg daily 2 wk and subsequently 200 mg thrice weekly for next 22 wk	Administration with food increases bioavailability; baseline ECG, then 2, 12, 24 wk after initiation to monitor QTc prolongation *esp*. in combination with clarithromycin, clofazimine and flouroquinolones; stop drug if QTc>500 ms; monitor serum calcium, magnesium and potassium	QTc prolongation, nausea, arthralgia, headache, subjective fever, anorexia

Contd...

Drug	Dosing	Monitoring	Serious adverse effects
Clofazimine (oral)	50-100 mg daily	ECG monitoring is required if used in combination with bedaquiline, flouroquinolones and macrolides (clarithromycin or azithromycin); monitor serum magnesium, potassium and calcium levels for QTc prolongation correct low levels before stopping the offending drugs ; not used in pregnancy and severe hepatic insufficiency; skin hyperpigmentation can prevented by applying sunscreen and lubricants	GI disturbances, dermatological discoloration: pink to brownish-black skin; discoloration appears within 4 wk and disappears after 6-10 months of the discontinuation, cornea, retina and urine; acne flare within 1-4 wk, ichthyosis and dry skin, QTc prolongation
Tobramycin (intravenous)	5-7 mg/kg per 24 h daily	Obtain peak level 2 and 6 h post dose until therapeutic goal back-extrapolated C_{max} 10 the tobramycin MIC, along with undetectable trough. Observe weekly CBC count, creatinine level, tobramycin troughs weekly (should remain <1.2 mg/ml); baseline and monthly audiograms and vestibular function tests	Nephrotoxicity, ototoxicity
Imipenem/cilastin (intravenous)	1g every 12 h (preferred). May consider 500 mg every 12 h for small, frail, or elderly patients	Monitor serum creatinine level, CBC count with differential count, ALT/AST levels weekly; dose adjustment required in CKD	GI disturbances, seizures, rash, cytopenia
Tigecycline (intravenous)	100 mg loading dose, subsequently 25-50 mg once daily (consider lower dose of 25 mg in case of intolerance to higher dosing)	Obtain serum creatinine level, CBC count, ALT/AST levels weekly; monitor INR and reduce warfarin dose	GI disturbances, hepatitis, prolonged aPTT, prolonged PT
Tedizolid (intravenous)	200 mg every 24 h	Monitor CBC count weekly 2 wk, then twice weekly	Myelosuppression, peripheral neuropathy, serotonin syndrome
Cefoxitin (intravenous)	Preferred: 1-2 g every 6-8 h Alternative: 3 g every 12 h	Weekly CBC count monitoring with differential count, creatinine level, and ALT level	Rash, neutropenia, thrombocytopenia

ALT, alanine transaminase; AST, aspartate transaminase; CBC, complete blood cell; CKD, chronic kidney disease; CNS, central nervous system; Crcl dB, decibel on audiogram; ECG, electrocardiogram; GI, gastrointestinal; LFT, liver function test; MAC, *Mycobacterium avium* complex; MIC, minimum inhibitory concentration; mo, months; PO, oral; TWI, three times per week; aPTT, activated partial thromboplastin time, PT, prothrombin time; NTM-PD, non-tuberculous mycobacterial pulmonary disease. *Source*: Refs 1, 17, 144, 190

available which can help in improving the quality of life in patients with NTM-PD. Chest physiotherapy can be helpful in improving lung functions and mucociliary clearance, especially in cavitary disease, CF and bronchiectasis. Breathing exercises including aerobic activity such as yoga are generally believed to be helpful in pulmonary rehabilitation[160]. Besides drug therapy, exposure to NTM, especially from household plumbing and water sources, should be avoided. NTM transmission can be prevented by increasing water temperature to ≥54°C (130°F) and changing shower heads regularly[10]. Patients with GERD should be advised to avoid foods that may trigger it and avoid vulnerable body positions that may cause repeated aspirations.

Patients should be advised to pay special attention to maintain adequate calorie intake and body mass index especially if surgical intervention is contemplated. Monitoring of pre-albumin level can serve as a useful marker of nutrition[184]. In some individuals along with antibiotic regimen, probiotic therapy can be helpful.

Box VII. Recommendations for treating and preventing disseminated *Mycobacterium avium* complex (MAC) disease
Treating Disseminated MAC Disease
Preferred therapy
At least 2 drugs as initial therapy to prevent or delay emergence of resistance
Clarithromycin 500 mg PO twice daily (AI) plus ethambutol 15 mg/kg PO daily or
Azithromycin 500-600 mg (AII) plus ethambutol 15 mg/kg PO daily when drug interactions or intolerance precludes the use of clarithromycin
Note: Testing of susceptibility to clarithromycin or azithromycin is recommended.
Alternative therapy
Some experts would recommend addition of a third or a fourth drug for people with HIV with high mycobacterial loads (*i.e.*, >2 log cfu/ml of blood), or in the absence of effective ART
The third or fourth drug options may include:
Rifabutin 300 mg PO daily (dose adjustment may be necessary based on drug-drug interactions) or A fluoroquinolone (*e.g.*, levofloxacin 500 mg PO daily or moxifloxacin 400 mg PO daily), or An injectable aminoglycoside (*e.g.*, amikacin 10-15 mg/kg iv daily or streptomycin 1 gm iv or im daily) Chronic maintenance therapy (secondary prophylaxis): Same as treatment regimens
Criteria for discontinuing chronic maintenance therapy
Completed at least 12 month therapy
No signs and symptoms of MAC disease
Have sustained (>6 months) CD4 count >100 cells/µl in response to ART
Indication for restarting secondary prophylaxis
CD4 <100 cells/µl
Other considerations
NSAIDs may be used for people with HIV who experience moderate to severe symptoms attributed to IRIS
If IRIS symptoms persist, a short-term course (four weeks-eight weeks) of systemic corticosteroid (equivalent to prednisone 20-40 mg) can be used
Preventing first episode of disseminated MAC disease (primary prophylaxis)
Primary prophylaxis is not recommended for adults and adolescents who immediately initiate ART. Indications for initiating primary prophylaxis
Not on fully suppressive ART, and
CD4 count
Preferred therapy
Azithromycin 1200 mg PO once weekly or Clarithromycin 500 mg PO BID or azithromycin 600 mg PO twice weekly
Alternative therapy
Rifabutin 300 mg PO daily (BI) (dose adjustment may be necessary based on drug-drug interactions)
Note: Active TB should be ruled out before starting rifabutin. Indication for discontinuing primary prophylaxis
Initiation of effective ART indication for restarting primary prophylaxis
CD4 count <50 cells/µl (only if not fully suppressive ART) ARTIII
ART: antiretroviral therapy, ARV, antiretroviral; BID, twice daily; CD4:CD4 T lymphocyte; cfu, colony-forming units; im, intramuscular; IRIS, immune reconstitution inflammatory syndrome; iv, intravenous; NSAIDs, non-steroidal anti-inflammatory drugs; PO, orally. *Source*: Ref. 19

Box VII details recommendations for preventing disseminated MAC disease[19] and includes indications for initiating, discontinuing and restarting primary prophylaxis. Disseminated MAC disease must be carefully ruled out before starting drugs for primary prophylaxis. While azithromycin (1200 mg PO once weekly) or clarithromycin (500 mg PO twice daily) are preferred drugs, rifabutin (300 mg PO daily) is an alternative drug for primary prophylaxis provided that the active TB has been ruled out.

Future prospects

Future studies should be directed to understand the role of risk factors for developing NTM-PD so that the benefit of screening can be offered to high risk individuals for early diagnosis and treatment. As growing evidence has established human-to-human transmission of *M. abscessus* among CF patients, further research should be done to study mechanisms contributing to patient-to-patient transmission of other NTM species to prevent further spread. Newer non-culture-based methods should be developed for early identification and speciation of NTM from respiratory specimens as the present methods rely heavily on mycobacterial culture for identification and further characterization of NTM species, causing significant delay in treatment. Future research should focus on understanding the role of DST in predicting treatment outcomes in NTM as currently the role of DST is controversial and limited only to a few situations in the management of NTM. Studies should also focus on understanding the pathogenic potential of various NTM species and subspecies to facilitate decision-making in treatment as there are significant knowledge gaps at present. Efforts should be made to follow progression of inflammatory lung disease systematically and to study treatment outcomes after timely intervention to develop and validate newer drugs and besides conventional routes of drug administration, potential use of drugs through inhalation route should be explored. Less toxic and more effective drug treatment regimens administered for short periods should be developed for the treatment of NTM-PD especially due to *M. abscessus* as these NTM species respond poorly to treatment with frequent relapses occurring after stopping the treatment.

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