

# Growing Challenges of Lung Infections with Non-tuberculous Mycobacteria in Immunocompromised Patients: Epidemiology and Treatment

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## Abstract

Non-tuberculous mycobacteria (NTM) are increasingly recognized as opportunistic pathogens in humans and animals, particularly affecting those with compromised immune systems. These bacteria encompass a diverse group of mycobacterial species that are responsible for a range of infections, with pulmonary and skin-related conditions being the most common. The rise in NTM infections in recent years is a growing concern for healthcare, highlighting the urgent need to improve our understanding of NTM epidemiology and treatment strategies. This article reviews the NTM species associated with lung infections in immunocompromised patients and underscores the critical importance of advancing diagnostic and therapeutic approaches. The review is based on a thorough analysis of scientific literature from databases such as PubMed, Scopus, and ScienceDirect, covering studies up to June 2024. Through this comprehensive analysis, the article aims to provide detailed insights into the complexities of NTM diseases and spur further research and innovation in combating these challenging infections.

## Keywords

Non-tuberculous mycobacteria · Lung infections · Immunodeficiency · atypical mycobacteria

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## 1. Introduction

Non-tuberculous mycobacteria (NTM) encompass numerous species that pose a serious threat to human health. NTM have emerged as opportunistic pathogens causing infections in both immunocompromised and immunocompetent individuals (Gopalaswamy et al. 2020; Chai et al. 2022). Advances in molecular diagnostics have accelerated the study of NTM species, enabling rapid and precise identification, which is crucial for timely treatment (Horne and Skerrett 2019). However, the genetic diversity of NTM species complicates understanding of their pathogenesis and resistance mechanisms (Johnson and Odell 2014; Mercaldo et al. 2023). The global burden of NTM infections is increasingly recognized, and the challenges posed by NTM require coordinated research and surveillance to address this growing public health threat.

## 2. Characteristics and Epidemiology of NTM

*Mycobacterium*, a genus within the Actinobacteria class, comprises over 196 species, including 24 subspecies, representing a diverse group of microorganisms with unique features

(Parte 2014). These rod-shaped, Gram-positive bacteria are predominantly saprophytes found in soil and water, although several species are notable pathogens in humans and animals (Mencarini et al. 2017; Desai and Hurtado 2021; Gunasingam 2022; Gu et al. 2023). NTM, first isolated by Pinner (1935), have been recognized as a distinct entity within the *Mycobacterium* genus, separate from the tuberculosis (TB)-causing strains. Known by various names, including mycobacteria other than TB and atypical mycobacteria, these organisms have garnered attention due to their ubiquitous presence and rising incidence worldwide (Ahmed et al. 2020). The Runyon classification, which categorizes NTM based on pigment production and growth rate, remains a cornerstone in understanding their diversity (Table 1) (Runyon 1959; Herdman and Steele 2004; Salvana et al. 2007; Abdalla et al. 2009; Tortoli 2014; Koh 2017; Tortoli et al. 2017; Sharma and Upadhyay 2020). NTM are prevalent in natural environments such as soil and water and colonize artificial water systems, posing a serious challenge to public health systems (Steglich et al. 2020). The capacity of NTM to form biofilms enhances their survival and complicates efforts to eradicate them from infected sites. Clinically, NTM is known to cause a spectrum of diseases, most notably lung infections and extrapulmonary conditions affecting the skin, soft tissues, bones, and lymph nodes (Prevots et al. 2010; Chin et al. 2020; Ratnatunga et al. 2020; Dahl et al. 2022; Bhanushali et al. 2023). Diagnosing and treating NTM infections are fraught with challenges, primarily due to their resistance to standard antibiotics and the need for long-term therapy.

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**Table 1.** Short characteristics of NTM groups [based on Runyon (1959); Herdman and Steele (2004); Salvana et al. (2007); Abdalla et al. (2009); Tortoli (2014); Koh (2017); Tortoli et al. (2017); Sharma and Upadhyay (2020)]

Classification	Growth rate	Characteristics	Common examples
Group 1	Slow-growing	Photochromogenic: Develop pigment when exposed to light.	<i>Mycobacterium kansasii</i> , <i>Mycobacterium marinum</i>
Group 2	Slow-growing	Scotochromogenic: Produce pigment in both light and darkness.	<i>Mycobacterium scrofulaceum</i> , <i>Mycobacterium szulgai</i> , <i>Mycobacterium goodii</i>
Group 3	Slow-growing	Non-photochromogenic: Do not produce pigments.	MAC, <i>Mycobacterium ulcerans</i>
Group 4	Fast-growing	May or may not produce colored colonies.	<i>Mycobacterium fortuitum</i> , <i>Mycobacterium abscessus</i> , <i>Mycobacterium chelonae</i>

MAC, *Mycobacterium avium* complex; NTM, non-tuberculous mycobacteria.

A noticeable global increase in incidence and spread characterizes the epidemiology of NTM infections, however, accurate estimates of incidence are difficult due to lack of required registration (Cowman et al. 2019; Thornton et al. 2021). The global incidence of NTM lung disease ranges from 2 to 14 cases per 100,000 persons per year (Griffith et al. 2007). In the USA, coverage ranges from 1.0 to 1.8 cases per 100,000 persons, while in the UK, coverage ranges from 4 to 6.1 cases per 100,000 between 2007 and 2012 (Prevots and Marras 2015; Sharma and Upadhyay 2020). In Japan, the incidence is 13.7 cases per 100,000 people, and in the Netherlands, it is about 5.3 cases per 100,000 (Hoefsloot et al. 2013; Morimoto et al. 2014). In Australia, the estimated incidence rate for all NTM increased 2.3-fold from 11.10/100,000 in 2001 to 25.88/100,000 in 2016 (Thomson et al. 2017, 2020). An interesting observation is the higher frequency of lung diseases caused by atypical mycobacteria in populations living in coastal areas. This has been suggested to be due to environmental factors such as soil composition, water exposure, climate changes, and increased environmental exposure to trace metals in surface waters (Dahl et al. 2022). Climate change has contributed to the spread of NTM, creating alterations in temperature and precipitation patterns, influencing soil composition and water quality, and creating favorable conditions for NTM growth (Chin et al. 2020; Blanc et al. 2021; Kambali et al. 2021; Dahl et al. 2022).

### 3. Brief Overview of NTM Associated with Respiratory Infections

#### 3.1. *Mycobacterium kansasii*

Identified in 1953, *M. kansasii* is one of the six most frequently isolated NTM species, often found in tap water. It is genetically diverse, with seven subtypes, of which subtype I is the most common in human infections (Griffith et al. 2007; Huang et al. 2020; Akram and Rawla 2024). In Poland, detection rates exceed 35% of all NTM isolations, which is higher than in Europe (5%) and worldwide (4%) (Bakula et al.

2018). *M. kansasii* is a slow-growing bacterium that develops at 32–42°C, with colonies becoming visible after 10 days at 37°C. It primarily causes lung disease, especially in people with previous lung disease or a history of TB, but can also infect lymph nodes, skin, and the musculoskeletal and genitourinary systems (Johnston et al. 2017).

#### 3.2. *Mycobacterium chimaera*

First recognized in 2004, *M. chimaera*, part of the *Mycobacterium avium* complex (MAC), is a slow-growing bacterium that thrives at 25–35°C, forming scotochromogenic, rough colonies after 6–8 weeks (Tortoli et al. 2004). It primarily causes respiratory infections, posing a risk to those with weakened immune systems. In healthcare, *M. chimaera* is often isolated from heating/cooling units used in cardiothoracic surgery, leading to serious infections such as prosthetic valve endocarditis. Symptoms are non-specific, and the incubation period ranges from 1 month to 72 months (Riccardi et al. 2020; Natanti et al. 2021). Phenotypic methods alone cannot distinguish *M. chimaera* from other MAC members. Identification techniques include biochemical testing, polymerase chain reaction (PCR), certain commercial mycobacterial detection assays, and advanced species identification tools like MALDI-TOF MS (Buchanan et al. 2020).

#### 3.3. *Mycobacterium abscessus*

*M. abscessus* is a rapidly growing NTM bacterium that was first isolated by Moore and Frerichs (1953) from the knee abscess of a 63-year-old woman. Like other NTM species, *M. abscessus* is ubiquitous in the environment, and can endure harsh, nutrient-limited environments that would be lethal to most competing microorganisms, such as in chlorinated water (e.g., home and hospital water supplies) (Lopeman et al. 2019). It divides into three subspecies: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii*, which can be identified only by molecular methods (e.g., based on PCR-reverse hybridization or by multiple gene sequencing (*hsp65*, *rpoB*, *erm*(41)) (Nie et al. 2014; Ruis et al. 2021;

Rodríguez-Temporal et al. 2023). The bacterium is known to cause lung infections, skin and soft tissue infections, osteomyelitis, and disseminated infections (Moral et al. 2019; Lee and Choi 2022; Rodríguez-Temporal et al. 2023; Watanabe et al. 2023; Waugh and Wajahat 2023). Infections caused by these bacteria are a growing public health challenge and primarily affect immunocompromised individuals, especially those with cystic fibrosis or chronic lung disease (Degiacomi et al. 2019; Schuurbijs et al. 2020; Boeck et al. 2022). Due to high resistance to antibiotics (e.g., macrolides—mediated by inducible synthesis of erythromycin ribosome methylase; aminoglycosides—mediated by spontaneous single mutations in the *rrs* gene encoding the 16S rRNA; fluoroquinolones—mediated by a nucleotide variation at the quinolone resistance determining region or resistance to most  $\beta$ -lactams associated with the genome of *Mycobacterium abscessus* complex (MABC) encodes a class A  $\beta$ -lactamase [BlaMab]), treatment can be very difficult and expensive, as it requires the simultaneous use of several drugs including moxifloxacin, amikacin, and cefoxitin (Victoria et al. 2021). *M. abscessus* is also known for forming biofilms, which can help the bacteria evade treatment (Dokic et al. 2021; Meliefste et al. 2024).

### 3.4. *Mycobacterium chelonae*

Identified in 1903, *M. chelonae* is a Runyon Group IV organism and occurs in soil, water, and hospitals (Delghandi et al. 2020). It grows best at 30–32°C, and colonies appear in 15 days. *M. chelonae* commonly causes skin and soft tissue infections, often following medical procedures. It can also lead to eye infections and, rarely, lung infections. The bacterium can cause serious illness, such as bacteremia and osteomyelitis, especially in immunocompromised individuals (Gutierrez and Somoskovi 2014; Akram and Rawla 2024). Symptoms range from localized skin abscesses in healthy individuals to widespread skin disease in those with weakened immune systems (Vega-Dominguez et al. 2020; Gaudêncio et al. 2021).

### 3.5. *Mycobacterium wolinskyi*

Identified in 1999, *M. wolinskyi* is a rapidly growing NTM that thrives at 30–35°C and develops colonies in 2–4 days (Brown et al. 1999). It occurs in water and soil and is associated with infections associated with medical devices and procedures. *M. wolinskyi* can cause bacteremia, peritonitis, and skin infections (Yoo et al. 2013; de Man et al. 2016). *M. wolinskyi* is part of the *M. smegmatis* group, which includes *M. smegmatis*, *M. goodii*, and *M. wolinskyi*. This bacterium is distinguished from its counterparts by its variable sensitivity to certain antibiotics, including macrolides, doxycycline, ciprofloxacin, and cefoxitin, and

notably, its resistance to tobramycin, setting it apart from other species within this group (Ariza-Heredia et al. 2011; Hernández-Meneses et al. 2021).

### 3.6. *Mycobacterium heckeshornense*

Discovered in 2000, *M. heckeshornense* is related to *M. xenopi* and grows at 37–45°C, forming scotochromogenic colonies in about 4 weeks (Roth et al. 2000; Chan et al. 2011). It causes syringomyelia and can infect immunocompromised individuals. It is sensitive to several antibiotics but is resistant to isoniazid and rifampicin (Roth et al. 2000). The bacterium can also cause extrapulmonary disease, such as lymphadenitis and disseminated infections.

### 3.7. *Mycobacterium arupense*

Isolated in 2006, *M. arupense* forms pale pink colonies after 3–4 weeks and grows well at 30°C (Cloud et al. 2006). It occurs in soil, water, and clinical sources, causing lung and joint infections, especially in immunocompromised individuals. It is sensitive to ethambutol, clarithromycin, and rifabutin, but resistant to rifampicin and other antibiotics, which is the basis of the treatment strategy (Abudaff and Beam 2017).

### 3.8. *Mycobacterium parakoreense*

First described in 2013, *M. parakoreense* grows at 37°C and forms rough, pigmented colonies after 4 weeks (Herdman and Steele 2004; Kim et al. 2013). It is related to *M. koreense* and *M. triviale* and is distinguished by its unique genetic sequences. *M. parakoreense* is sensitive to amikacin, clarithromycin, and rifampicin, which allows it to be used in the treatment of infections (Kim et al. 2013).

### 3.9. *Mycobacterium persicum*

Identified in 2017, *M. persicum* grows as photochromogenic colonies at 37°C, related to *M. kansasii* (Herdman and Steele 2004). Detected in lung samples from Iranian patients, it is sensitive to amikacin, clarithromycin, and linezolid, but resistant to ethambutol (Shahraki et al. 2017). Restriction fragment length polymorphism analysis further supports its classification as a novel member of the *M. kansasii* complex, which includes *M. kansasii* and *M. gastri* (Shahraki et al. 2017).

### 3.10. *Mycobacterium basiliense*

Discovered in 2019, *M. basiliense* grows as non-photochromogenic colonies at 30°C (Seth-Smith et al. 2019). Isolated from respiratory samples, it affects both healthy and

immunocompromised individuals. It is susceptible to a range of antibiotics, including clarithromycin and rifampicin, offering multiple treatment options. While it shares phenotypic similarities with *M. marinum*, *M. basiliense* is capable of growing at 37°C, distinguishing it from other related species. Further differentiation comes from the analysis of cell wall mycolic acids through high-performance liquid chromatography, which revealed a distinct pattern compared with the closely related *M. marinum*.

### 3.11. MAC

MAC infections significantly affect respiratory health, especially in people with lung disease or weakened immune systems. MAC includes *M. avium* and *M. intracellulare*, which are common in the environment (Loebinger 2017). These non-motile, non-spore-forming, Gram-positive, acid-fast bacilli grow slowly, usually needing 10–20 days to form mature colonies. They are classified in class III in Runyon's classification. *M. avium* grows best at around 34.5°C, while *M. intracellulare* prefers around 31.5°C. Both can grow between 28°C and 38.5°C, with *M. avium* able to survive temperatures up to 49°C. This ability to adapt to temperature contributes to their resilience and persistence in the environment (Akram and Rawla 2024). In susceptible populations, such as patients with chronic obstructive pulmonary disease (COPD), cystic fibrosis or those undergoing immunosuppressive therapies, MAC can lead to a chronic lung disease known as MAC lung disease (Shin and Shin 2021). This disease process involves colonization of the airways by bacteria, followed by local immune invasion and granuloma formation (Koh et al. 2017). Diagnosis is usually based on clinical symptoms, radiological findings, and microbiological evidence of MAC from sputum or tissue samples. Treatment of MAC infections is difficult and requires prolonged courses of multiple antibiotics to effectively manage the infection. The mainstays of therapy are macrolides, rifamycin, and ethambutol. However, treatment efficacy varies and there is a significant potential for recurrence or reinfection (Kim et al. 2022).

## 4. Risk Factors, Pathogenesis, and Immune Response in Pulmonary NTM Infections

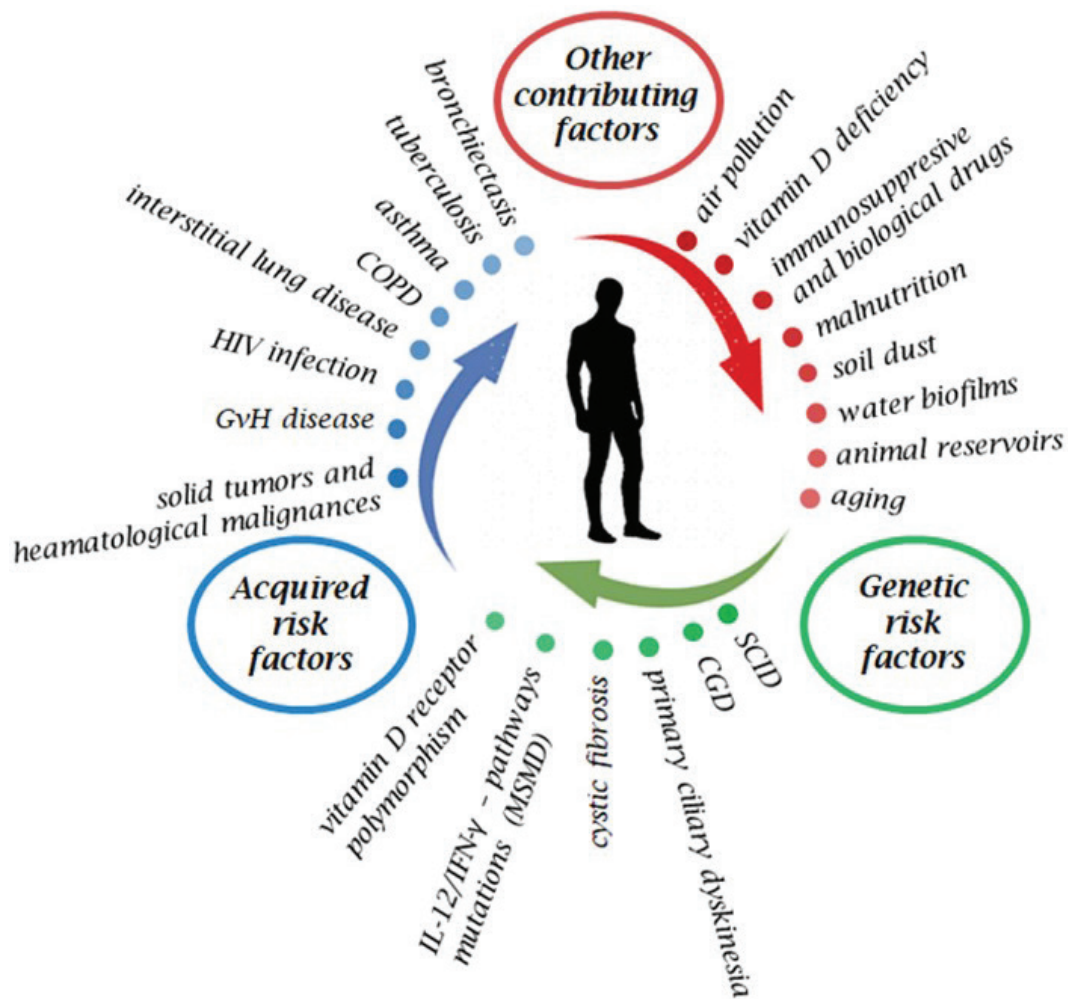
NTM infections are influenced by host-related and environmental factors, manifesting mainly as pulmonary or disseminated infections in immunocompromised individuals (Figure 1). Pulmonary NTM infections are often linked to lung impairments from conditions like bronchiectasis, COPD, emphysema, or cancer (Kumar et al. 2024). Genetic predispositions include cystic fibrosis,  $\alpha$ -1-antitrypsin deficiency, interferon (IFN)- $\gamma$  and interleukin (IL)-12

receptor anomalies, primary ciliary dyskinesia, and pulmonary alveolar proteinosis (Park et al. 2022). Non-genetic factors such as medications (anti-tumor necrosis factor [TNF]- $\alpha$  agents, cytotoxic drugs, corticosteroids), gastroesophageal reflux disease, vitamin D deficiency, rheumatoid arthritis, and allergic bronchopulmonary aspergillosis also contribute. Lady Windermere syndrome, affecting postmenopausal women, is another risk factor (Loebinger et al. 2023). Acquired immune deficiencies, such as HIV, hematologic malignancies, and autoantibodies against IFN- $\gamma$ , increase mycobacteriosis risk. Environmental factors include natural settings (soil, water) and artificial ones (hot tubs, hospital facilities) harboring contaminated water and equipment. NTM's lipid-rich cell walls enable biofilm formation, resisting antibiotics and disinfectants. Their specialized growth requirements often lead to underdiagnosis in routine tests (Antczak et al. 2017; Cowman et al. 2019; Pereira et al. 2020).

NTM diseases manifest in forms ranging from lymph node infections to symptoms resembling aseptic meningitis. The most common manifestation is lung infections, known as non-tuberculous mycobacterial pulmonary disease (NTM-PD), predominantly caused by MAC species and *M. abscessus* globally (Chotmongkol et al. 2024). NTM lung diseases exhibit three primary patterns based on their pathology: TB-like diseases with or without lung cavitation typically in older male smokers with COPD; bronchiectasis, often in slender, non-smoking older women known as "Lady Windermere syndrome," and lung inflammation resulting from continuous exposure to mycobacteria in water systems like those in residential, office, and health-care settings (Arend et al. 2009). Patients usually present with mixed forms of these patterns, making typical classification challenging.

The pathogenesis of NTM-PD is complex, taking months or years to develop, complicating diagnosis and often making it hard to trace the infection source (Wilińska and Szturmowicz 2010; Ratnatunga et al. 2020). Bacteria enter via the respiratory system, colonizing bronchial epithelial cells (Figure 2). The host activates airway clearance mechanisms, but if bacteria persist, they face the innate and acquired immune responses. Fibronectin-binding proteins on bacterial surfaces allow adhesion to the respiratory epithelium via integrin receptors (Honda et al. 2015). Colonizing mycobacteria undergo phenotypic changes, enhancing macrophage conquest. Their ability to inhibit inflammatory cytokine production and form biofilms compromise the immune response, allowing colonization and invasion of bronchial epithelial cells. Mycobacteria present in the macrophages can be killed or maintained within them by inducing NTM genes, inhibiting macrophage functions, lymphocyte proliferation, and causing macrophage destruction and apoptosis (Honda et al. 2015). Activated

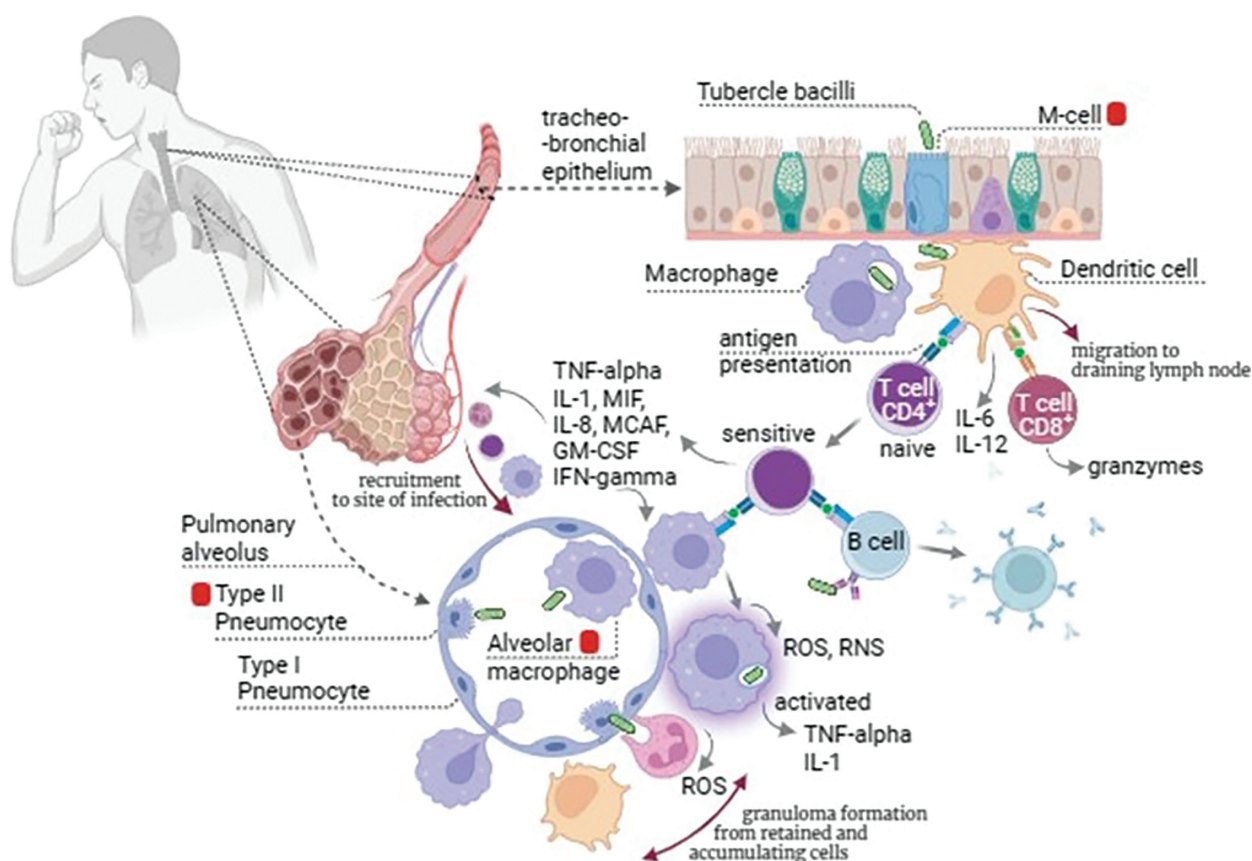




**Fig 1.** Risk factors contributing to NTM infections. The risk factors for NTM infection can be broadly categorized into environmental exposures, underlying health conditions, lifestyle factors, and certain procedural or occupational hazards. CGD, chronic granulomatous disease; COPD, chronic obstructive pulmonary disease; GvH, graft versus host; IFN, interferon; IL, interleukin; NTM, non-tuberculous mycobacteria; SCID, severe combined immunodeficiency.

macrophages produce reactive oxygen and nitrogen species against mycobacteria. Inflammatory response results from cytokine production by activated macrophages and secretion of IL-12 leads to T cell differentiation into Th1 cells. Macrophages also stimulate natural killer (NK) cells with cytotoxic properties. Dendritic cells, M cells, and neutrophils also participate in phagocytosis (Horne and Skerrett 2019). During a specific immune response, T lymphocytes recognize bacterial antigens and transform into effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells (Nair et al. 2016; Ndlovu and Marakalala 2016; Torrelles and Schlesinger 2017; Li et al. 2021). Antigens presented by antigen-presenting cells through MHC class II molecules are recognized by CD4<sup>+</sup> Th1 lymphocytes, producing IFN- $\gamma$  to enhance antigen presentation and macrophage killing. CD8<sup>+</sup> Tc (cytotoxic) lymphocytes recognize antigens through MHC class I

molecules, leading to apoptosis of infected host cells. Tc lymphocytes release cytokines like IFN- $\gamma$ , mediating the immune response.  $\gamma/\delta$  T lymphocytes also recognize antigens without MHC C involvement (Shu et al. 2020). When a pathogen evades the host's immune response, it can persist long-term. For *M. tuberculosis*, mechanisms enabling persistence include granuloma formation, which contain the bacteria and prevent clearance, and enter a dormant state to evade immune surveillance. *M. tuberculosis* also manipulates the host's immune response by modulating cytokine and chemokine production, influencing granuloma macrophages to undergo transformation to other morphological forms conducive to bacterial survival (Etna et al. 2014; Guler et al. 2021). Granulomas consist of a collection of organized immune cells, and using modern genetic techniques (e.g., parallel ssRNAseq), a high



**Fig 2.** Immune mechanisms accompanying mycobacterial infection. GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; MCAF, monocyte chemotactic and activating factor; MIF, migration inhibitory factor; RNS, reactive nitrogen species; ROS, reactive oxygen species; TNF, tumor necrosis factor.

degree of heterogeneity between different granulomas has been shown (Cooper et al. 2024). Host-pathogen interactions modulate the processes leading to the formation of a wide spectrum of granuloma structures even within a single human host. From a histological point of view, in TB frequently the most central part of the granuloma is an acellular caseous necrotic region containing mycobacteria, while it is also possible to form other types of granulomas, which may not have a necrotic area and are composed primarily of macrophages and a few lymphocytes (Guirado and Schlesinger 2013). Macrophages play a key role in the formation of granulomas, and they represent a significant amount of the granuloma cell population, forming the inner layers of the granuloma and acting as a central skeleton that facilitates the organization of other cell types around it. Macrophages undergo “epithelial differentiation,” tightly assembling together to form the granuloma. Epithelial macrophages are a key component of tuberculoid granulomas, accompanied by “conventional” macrophages, foam macrophages, and multinucleated giant cells (Cronan 2022). Other cell types are also identified in the granuloma structure: neutrophils, dendritic cells, eosinophils, mast cells,

T and B lymphocytes, NK cells and innate lymphoid cells, and even fibroblasts, and endothelial and epithelial cells, most of which are found in the periphery of the granuloma (Cronan 2022; Weeratunga et al. 2024). NTM mycobacteria are less virulent than *M. tuberculosis* but can pose serious threats to immunocompromised individuals, such as those with HIV/AIDS or undergoing immunosuppressive therapy (Varma-Basil and Bose 2019). Studies on human leukemia monocytic cell line Tohoku Hospital Pediatrics-1 (THP-1) infected with various NTM strains show that NTMs adopt different strategies to manipulate host defenses for long-term persistence (Sousa et al. 2019). Key factors include phagosome acidification, nitric oxide (NO) production, and cell death. Some strains are cleared within 24 h (*M. smegmatis* and *M. fortuitum*), while others replicate (*M. avium* and *M. fortuitum*). A study using human cells (THP-1, monocyte-derived macrophages, and alveolar macrophages) infected with various NTM found that macrophage infectivity and virulence vary among NTM species and isolates. Some NTMs evade antibacterial peptides like LL-37 through modified phospholipids, representing a novel virulence mechanism (Honda et al. 2020).

## 5. Diagnosis and Therapy of Pulmonary NTM Infections—A Perspective for Effective Disease Control

In 2007, the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) issued a statement outlining the criteria for the diagnosis and treatment of pulmonary diseases related to NTM-PD (Griffith et al. 2007; Daley et al. 2020a). According to these guidelines, the diagnosis is based on clinical symptoms, radiological findings, and microbiological results. Clinical indicators include respiratory symptoms (chronic or recurrent cough, sputum production, shortness of breath) and systemic symptoms (fatigue, weight loss, fever, chest pain). Radiological confirmation of infection is required through chest X-rays showing nodular or cavitary opacities, or high-resolution computer tomography showing bronchiectasis with numerous small nodules. Microbiological criteria include at least two positive culture results from independent sputum samples or one positive culture from bronchial washings, bronchoalveolar lavage, or histopathological examination with a positive lung biopsy, and at least one sputum or bronchial wash positive for NTM in culture (Griffith et al. 2007; Daley et al. 2020a). Identification mainly relies on acid-fast bacilli staining (e.g., Ziehl-Neelsen method and fluorescence) and testing using molecular diagnostic tools like GeneExpert. Samples are cultured on media suitable for NTM growth: Löwenstein–Jensen, Middlebrook, and Dubos (Sharma and Upadhyay 2020). NTM species identification uses biochemical tests, now being phased out for modern techniques. High-performance liquid chromatography identifies slow-growing NTM but is less specific for rapidly growing species. Molecular methods have revolutionized identification, including Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), real-time PCR, gene probes, linear probe hybridization, DNA sequencing, and Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF-MS). Several readymade DNA sequencing systems are available, but some species are genetically similar, complicating diagnosis. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF)'s effectiveness also depends on the available sequence library (Pennington et al. 2021).

Treatment of NTM infections is complex and involves prolonged multidrug regimens, ranging from 3 months to 4 months for skin and soft tissue infections to over 12 months for lung and disseminated infections. Treatment often results in significant side effects and is poorly tolerated, especially in patients with coexisting diseases. New drugs used in multidrug-resistant TB, such as bedaquiline, pretomanid, and delamanid, are being tested for effectiveness against NTM. Guidelines for managing NTM-PD follow recommendations from ATS/European Respiratory Society (ERS)/European Society of Clinical Microbiology and Infectious Diseases (ESCMID)/

IDSA, which are based on expert opinions, observational studies, and controlled trials, as well as the British Thoracic Society (Haworth et al. 2017a; Daley et al. 2020b). Antibiotic therapy is the primary treatment approach, with regimens tailored based on the NTM species, disease severity, and drug susceptibility results (Pathak et al. 2022). For slow-growing mycobacteria, a typical regimen includes rifampicin or rifabutin, ethambutol, and a macrolide for 12 months, with amikacin or streptomycin added for severe cases during the first 3 months (Larsson et al. 2017). Treatment for fast-growing mycobacteria depends on *in vitro* sensitivity tests. Recovery rates from these infections vary from 34% to 65%, with recurrence rates of up to 48% (Taylor and Mitchell 2023). Current guidelines recommend continuing antibiotic therapy for at least 12 months after a negative culture conversion to minimize recurrence risk (Shulha et al. 2019; Daley et al. 2020c). If antibiotic response is poor, if there is macrolide resistance, or if severe symptoms like coughing up blood occur, surgical treatment is advised (Desai and Hurtado 2017; Haworth et al. 2017b). Surgery is used in NTM pulmonary disease management to improve cure rates, especially in patients with focal lesions, and must be preceded by thorough radiological examination and/or biopsy (Lu et al. 2018). NTM infections, primarily affecting the lungs (80%–90% of cases), show varied lesion images on computed tomography scans and X-rays, influenced by the patient's immune status and preexisting lung diseases (Jamal and Hammer 2022). Surgical outcomes differ by institution. For example, a study from Seoul National University Hospital reported postoperative complications in 13.4% of NTM-PD patients, with adverse outcomes linked to factors such as female gender and preoperative positive mycobacterial cultures. In contrast, a study from Osaka, Japan, noted a 20% adverse outcome rate with a high rate of negative sputum culture conversion (Fukushima et al. 2020; Kim et al. 2021, 2023). Addressing antibiotic resistance is crucial for managing NTM-PD, as resistant strains lead to low culture conversion rates and high 5-year mortality (van der Laan et al. 2022). An innovative approach in NTM-LD therapy involved using three bacteriophage strains to treat *M. abscessus* infection in a teenager with cystic fibrosis at Great Ormond Street Hospital in London. Phage therapy successfully eliminated the pathogen without cytotoxic effects, unlike traditional chemotherapeutics. However, phage therapy has significant limitations. Bacteriophages are highly specific to particular bacterial strains, requiring the screening of over 10,000 phage strains, with two genetically modified, to find three actives against *M. abscessus*. The therapy did not yield the same results against different *M. abscessus* strains in other patients. Thus, while phage therapy shows promise, its need for a highly individualized approach limits its widespread use in the near future (Dedrick et al. 2019; Laudone et al. 2021). An interesting solution is using liposomes as carriers for antibiotics, addressing the poor penetration of drugs into macrophages and cells targeted by pathogens and biofilms, including NTM.

While liposomes are widely used for various applications, they have not been extensively used in mycobacterial infection therapy. The only approved liposomal therapeutic for treatment-resistant *Mycobacterium avium* complex pulmonary disease (MAC-PD) in the USA, EU, and Japan is a liposomal inhalation suspension of amikacin (ALIS). ALIS, composed of dipalmitoylphosphatidylcholine and cholesterol, is designed to provide strong antibacterial activity against MAC, targeted delivery to infection sites, and penetration into intracellular spaces, including macrophages and biofilms (Shirley 2019; Hoy 2021; Winthrop et al. 2021; Morimoto et al. 2024).

The search for new chemotherapeutic agents with antitubercular effects is a direct approach. Several new antibiotics, including novel benzimidazoles like SPR719 and EJMCh-6, have shown potency in lab tests against various NTM species (e.g., *M. ulcerans*, *M. marinum*, *M. chimaera*, *M. avium*, *M. abscessus*) (Pidot et al. 2021; Quang and Jang 2021; Aragaw et al. 2022). Studies on SPR720 for MAC-PD have entered phase 2a trials but were suspended by the U.S. Food and Drug Administration. Additionally, delamanid and pretomanid, two new anti-TB drugs, are being considered for *M. abscessus*, though further research is needed to confirm their effectiveness (Kumar et al. 2022). Investigating drug administration through inhalation offers an alternative to antibiotics, such as NO or granulocyte-macrophage colony-stimulating factor (GM-CSF). Studies with NTM-PD patients showed that inhaling 160 ppm of NO for 50 min, three times daily, 5 days a week for 3 weeks, improved clinical symptoms and reduced NTM presence in sputum cultures (Flume et al. 2023). Inhaling GM-CSF could also be beneficial for NTM therapy. Macrophages activated by GM-CSF can better combat NTM. Monocyte-derived macrophages in bronchiectatic airways are less effective at killing NTM compared with resident alveolar macrophages due to limited GM-CSF exposure. Treatment with GM-CSF may enhance the ability of these macrophages to eliminate NTM, even when standard antibiotics fail (Hisert et al. 2023). In 2023, the clinical trial with 32 NTM-PD patients treated with inhaled GM-CSF (300 µg/day for 48 weeks) showed a 25% culture conversion rate, mainly in *M. avium* complex cases (Thomson et al. 2023).

The role of the *M. bovis* Bacillus Calmette-Guérin (BCG) vaccine in NTM therapy remains under investigation, aiming to establish long-lived memory T cells. Before modern HIV treatments, disseminated infections from NTM, especially *M. avium* complex, were more frequent, highlighting the role of CD4<sup>+</sup> T cells in defending against mycobacterial infections (Verma et al. 2020). In studies on immunocompromised mice, the BCG vaccine reduced the bacterial burden in the lungs and spleen. DNA plasmids and recombinant BCG vaccines encoding specific mycobacterial genes have shown promising effects against *M. avium* (Orujyan et al. 2022). Abate et al. (2019) demonstrated that BCG immunization induces

cross-reactive T cells inhibiting intracellular replication of *M. avium* and *M. abscessus*, increasing IL-17 and IFN- $\gamma$  production. However, a study with 1000 participants at Seoul National University Hospital found that BCG was not protective against NTM-PD progression (Kwak et al. 2022). This might be influenced by the number of BCG doses. In most countries, only one dose is given shortly after birth, or revaccination is carried out at a later age, as was the case in South Korea, Singapore, Taiwan, and Malaysia; however, these countries withdrew from this practice now (Tam and Leung 2000). Meanwhile, in Turkey, full immunization includes up to five doses: at birth, at 2 months after birth, at 6–7 years of age, at 11–12 years of age, and at 16–17 years of age (Verma et al. 2020). Further research is therefore necessary, both *in vitro* and cohort studies, which will improve our knowledge on how BCG vaccination affects NTM infections/outcome and the persistence of NTM in the human host.

## 6. Conclusions

The increasing prevalence of NTM infections requires a comprehensive understanding of their epidemiology, etiology, and clinical manifestations. Current diagnostic methods often fail to accurately identify NTM infections, delaying treatment and leading to poor outcomes. Thus, developing more sensitive and specific diagnostic tools is critical for early detection. Treating NTM infections also presents significant challenges. Current regimens, while sometimes effective, often fail and are associated with side effects. Drug resistance further threatens treatment efficacy, underscoring the urgent need for novel therapeutics that improve efficacy and reduce adverse effects. Future research should prioritize understanding NTM pathogenesis and drug resistance mechanisms and identifying new therapeutic targets.

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## Author Contributions

Conceptualization, M.D. and W.B. software, W.B.; writing—original draft preparation, M.D., W.B., M.F.; writing—review and editing, M.D, W.B, M.F.; visualization, W.B., M.F; supervision, M.D. All authors have read and agreed to the published version of the manuscript.

## Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript, or in the decision to publish the results.



## References

- Abate G, Hamzabegovic F, Eickhoff CS et al. (2019) BCG vaccination induces *M. avium* and *M. abscessus* cross-protective immunity. *Front Immunol* 10:234. <https://doi.org/10.3389/fimmu.2019.00234>
- Abdalla CM, de Oliveira ZN, Sotto MN et al. (2009) Polymerase chain reaction compared to other laboratory findings and to clinical evaluation in the diagnosis of cutaneous tuberculosis and atypical mycobacteria skin infection. *Int J Dermatol* 48:27–35. <https://doi.org/10.1111/j.1365-4632.2009.03807.x>
- Abudaff NN, Beam E (2017) *Mycobacterium arupense*: A review article on an emerging potential pathogen in the *Mycobacterium terae* complex. *J Clin Tuberc Other Mycobact Dis* 10:1–5. <https://doi.org/10.1016/j.jctube.2017.11.001>
- Ahmed I, Tiberi S, Farooqi J et al. (2020) Non-tuberculous mycobacterial infections – A neglected and emerging problem. *Int J Infect Dis* 92S:S46–S50. <https://doi.org/10.1016/j.ijid.2020.02.022>
- Akram SM, Rawla P (2024) *Mycobacterium kansasii* infection. StatPearls Publishing, Treasure Island, FL. <https://www.ncbi.nlm.nih.gov/books/NBK430906/>
- Antczak M, Dadura K, Lewandowska K, Dziadek J (2017) [Nontuberculous mycobacteria – Why treatment is so difficult?]. *Kosmos* 66:31–40.
- Aragaw WW, Cotroneo N, Stokes S et al. (2022) In vitro resistance against DNA gyrase inhibitor SPR719 in *Mycobacterium avium* and *Mycobacterium abscessus*. *Microbiol Spectr* 10:e0132121. <https://doi.org/10.1128/spectrum.01321-21>
- Arend SM, van Soolingen D, Ottenhoff TH (2009) Diagnosis and treatment of lung infection with nontuberculous mycobacteria. *Curr Opin Pulm Med* 15:201–208. <https://doi.org/10.1097/MCP.0b013e3283292679>
- Ariza-Heredia EJ, Dababneh AS, Wilhelm MP et al. (2011) *Mycobacterium wolinskyi*: A case series and review of the literature. *Diagn Microbiol Infect Dis* 71:421–427. <https://doi.org/10.1016/j.diagmicrobio.2011.08.005>
- Bakula Z, Kościuch J, Safianowska A et al. (2018) Clinical, radiological and molecular features of *Mycobacterium kansasii* pulmonary disease. *Respir Med* 139:91–100. <https://doi.org/10.1016/j.rmed.2018.05.007>
- Bhanushali J, Jadhav U, Ghewade B et al. (2023) Unveiling the clinical diversity in nontuberculous mycobacteria (NTM) infections: A comprehensive review. *Cureus* 15:e48270. <https://doi.org/10.7759/cureus.48270>
- Blanc SM, Robinson D, Fahrenfeld NL (2021) Potential for nontuberculous mycobacteria proliferation in natural and engineered water systems due to climate change: A literature review. *City Environ Interact* 11:100070. <https://doi.org/10.1016/j.cacint.2021.100070>
- Boeck L, Burbaud S, Skwark M et al. (2022) *Mycobacterium abscessus* pathogenesis identified by phenogenomic analyses. *Nat Microbiol* 7:1431–1441. <https://doi.org/10.1038/s41564-022-01204-x>
- Brown BA, Springer B, Steingrube VA et al. (1999) *Mycobacterium wolinskyi* sp. nov. and *Mycobacterium goodii* sp. nov., two new rapidly growing species related to *Mycobacterium smegmatis* and associated with human wound infections: A cooperative study from the International Working Group on Mycobacterial Taxonomy. *Int J Syst Bacteriol* 49(Pt 4):1493–1511. <https://doi.org/10.1099/00207713-49-4-1493>
- Buchanan R, Agarwal A, Mathai E et al. (2020) *Mycobacterium chimaera*: A novel pathogen with potential risk to cardiac surgical patients. *Natl Med J India* 33:284–287. <https://doi.org/10.4103/0970-258X.317473>
- Chai J, Han X, Mei Q et al. (2022) Clinical characteristics and mortality of non-tuberculous mycobacterial infection in immunocompromised vs. immunocompetent hosts. *Front Med (Lausanne)* 9:884446. <https://doi.org/10.3389/fmed.2022.884446>
- Chan WW, Murray MC, Tang P et al. (2011) *Mycobacterium heckeshornense* peritonitis in a peritoneal dialysis patient: A case report and review of the literature. *Clin Microbiol Infect* 17:1262–1264. <https://doi.org/10.1111/j.1469-0691.2010.03449>
- Chin KL, Sarmiento ME, Alvarez-Cabrera N et al. (2020) Pulmonary non-tuberculous mycobacterial infections: Current state and future management. *Eur J Clin Microbiol Infect Dis* 39:799–826. <https://doi.org/10.1007/s10096-019-03771-0>
- Chotmongkol V, Kosallavat S, Sawanyawisuth K et al. (2024) Evaluation of seegeneanyplex MTB/NTM real-time detection assay for diagnosis of tuberculous meningitis. *Orphanet J Rare Dis* 19:7. <https://doi.org/10.1186/s13023-023-03009-5>
- Cloud JL, Meyer JJ, Pounder JI et al. (2006) *Mycobacterium arupense* sp. nov., a non-chromogenic bacterium isolated from clinical specimens. *Int J Syst Evol Microbiol* 56:1413–1418. <https://doi.org/10.1099/ijs.0.64194-0>
- Cooper SK, Ackart DF, Lanni F et al. (2024) Heterogeneity in immune cell composition is associated with *Mycobacterium tuberculosis* replication at the granuloma level. *Front Immunol* 15:1427472. <https://doi.org/10.3389/fimmu.2024.1427472>
- Cowman S, van Ingen J, Griffith DE et al. (2019) Non-tuberculous mycobacterial pulmonary disease. *Eur Respir J* 54:1900250. <https://doi.org/10.1183/13993003.00250-2019>
- Cronan MR (2022) In the thick of it: Formation of the tuberculous granuloma and its effects on host and therapeutic responses. *Front Immunol* 13:820134. <https://doi.org/10.3389/fimmu.2022.820134>
- Dahl VN, Mølhave M, Fløe A et al. (2022) Global trends of pulmonary infections with nontuberculous mycobacteria: A systematic review. *Int J Infect Dis* 125:120–131. <https://doi.org/10.1016/j.ijid.2022.10.013>
- Daley CL, Iaccarino JM, Lange C et al. (2020a) Treatment of non-tuberculous mycobacterial pulmonary disease: An official ATS/ERS/ESCMID/IDSA clinical practice guideline. *Clin Infect Dis* 71:905–913. <https://doi.org/10.1093/cid/ciaa1125>
- Daley CL, Iaccarino JM, Lange C et al. (2020b) Treatment of non-tuberculous mycobacterial pulmonary disease: An official ATS/

- ERS/ESCMID/IDSA clinical practice guideline. Clin Infect Dis 71:e1–e36. <https://doi.org/10.1093/cid/ciaa241>
- Daley CL, Iaccarino JM, Lange C et al. (2020c) Treatment of non-tuberculous mycobacterial pulmonary disease: An official ATS/ERS/ESCMID/IDSA clinical practice guideline. Eur Respir J 56:2000535. <https://doi.org/10.1183/13993003.00535-2020>
- de Man TJ, Perry KA, Lawsin A et al. (2016) Draft genome sequence of *Mycobacterium wolinskyi*, a rapid-growing species of non-tuberculous mycobacteria. Genome Announc 4:e138–e116. <https://doi.org/10.1128/genomeA.00138-16>
- Dedrick RM, Guerrero-Bustamante CA, Garlena RA et al. (2019) Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. Nat Med 25:730–733. <https://doi.org/10.1038/s41591-019-0437-z>
- Degiacomi G, Sammartino JC, Chiarelli LR et al. (2019) *Mycobacterium abscessus*, an emerging and worrisome pathogen among cystic fibrosis patients. Int J Mol Sci 20:5868. <https://doi.org/10.3390/ijms20235868>
- Delghandi MR, El-Matbouli M, Menanteau-Ledouble S (2020) Mycobacteriosis and infections with non-tuberculous mycobacteria in aquatic organisms: A review. Microorganisms 8:1368. <https://doi.org/10.3390/microorganisms8091368>
- Desai AN, Hurtado R (2021) Nontuberculous mycobacterial infections. The Journal of the American Medical Association (JAMA) 325(15):1574. <https://doi.org/10.1001/jama.2020.19062>
- Dokic A, Peterson E, Arrieta-Ortiz ML et al. (2021) *Mycobacterium abscessus* biofilms produce an extracellular matrix and have a distinct mycolic acid profile. Cell Surf 7:100051. <https://doi.org/10.1016/j.tcsu.2021.100051>
- Etna MP, Giacomini E, Severa M et al. (2014) Pro- and anti-inflammatory cytokines in tuberculosis: A two-edged sword in TB pathogenesis. Semin Immunol 26:543–551. <https://doi.org/10.1016/j.smim.2014.09.011>
- Flume PA, Garcia BA, Wilson D et al. (2023) Inhaled nitric oxide for adults with pulmonary non-tuberculous mycobacterial infection. Respir Med 206:107069. <https://doi.org/10.1016/j.rmed.2022.107069>
- Fukushima K, Miki M, Matsumoto Y et al. (2020) The impact of adjuvant surgical treatment of nontuberculous mycobacterial pulmonary disease on prognosis and outcome. Respir Res 21:153. <https://doi.org/10.1186/s12931-020-01420-1>
- Gaudêncio M, Carvalho A, Bertão MI et al. (2021) *Mycobacterium chelonae* cutaneous infection: A challenge for an internist. Eur J Case Rep Intern Med 8:003013. [https://doi.org/10.12890/2021\\_003013](https://doi.org/10.12890/2021_003013)
- Gopalaswamy R, Shanmugam S, Mondal R et al. (2020) Of tuberculosis and non-tuberculous mycobacterial infections – A comparative analysis of epidemiology, diagnosis and treatment. J Biomed Sci 27:74. <https://doi.org/10.1186/s12929-020-00667-6>
- Griffith DE, Aksamit T, Brown-Elliott BA et al. (2007) An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 175:367–416. <https://doi.org/10.1164/rccm.200604-571ST>
- Gu Y, Nie W, Huang H et al. (2023) Non-tuberculous mycobacterial disease: Progress and advances in the development of novel candidate and repurposed drugs. Front Cell Infect Microbiol 13:1243457. <https://doi.org/10.3389/fcimb.2023.1243457>
- Guirado E, Schlesinger LS (2013) Modeling the *Mycobacterium tuberculosis* granuloma – The critical battlefield in host immunity and disease. Front Immunol 4:98. <https://doi.org/10.3389/fimmu.2013.00098>
- Guler R, Ozturk M, Sabeel S et al. (2021) Targeting molecular inflammatory pathways in granuloma as host-directed therapies for tuberculosis. Front Immunol 12:733853. <https://doi.org/10.3389/fimmu.2021.733853>
- Gunasingam N (2022) Morphology and pathological characteristics of mycobacteria. Mycobact Dis S4:005. <https://doi.org/10.35248/2161-1068.22.S4.005>
- Gutierrez C, Somoskovi A (2014) Human pathogenic mycobacteria. Ref Module Biomed Sci. Elsevier. <https://doi.org/10.1016/B978-0-12-801238-3.00137-9>
- Haworth CS, Banks J, Capstick T et al. (2017a) British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). Thorax 72(Suppl. 2):ii1–ii64. <https://doi.org/10.1136/thoraxjnl-2017-210927>
- Haworth CS, Banks J, Capstick T et al. (2017b) British Thoracic Society Guideline for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). BMJ Open Respir Res 4:e000242. <https://doi.org/10.1136/bmjresp-2017-000242>
- Herdman AV, Steele JC Jr (2004) The new mycobacterial species – Emerging or newly distinguished pathogens. Clin Lab Med 24:651–690. <https://doi.org/10.1016/j.cll.2004.05.011>
- Hernández-Meneses M, González-Martin J, Agüero D et al. (2021) Hospital clinic of Barcelona infectious endocarditis team. *Mycobacterium wolinskyi*: A new non-tuberculous *Mycobacterium* associated with cardiovascular infections? Infect Dis Ther 10:1073–1080. <https://doi.org/10.1007/s40121-021-00416-8>
- Hisert KB, Ochoa A, Corley J et al. (2023) GM-CSF is essential for effective macrophage killing of nontuberculous mycobacteria. Am J Respir Crit Care Med 207:A4240. <https://doi.org/10.1164/ajrccm-conference.2023.207.1>
- Hoefsloot W, van Ingen J, Andrejak C et al. (2013) The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: An NTM-NET collaborative study. Eur Respir J 42:1604–1613. <https://doi.org/10.1183/09031936.00149212>
- Honda JR, Hess T, Carlson R et al. (2020) Nontuberculous mycobacteria show differential infectivity and use phospholipids to antagonize LL-37. Am J Respir Cell Mol Biol 62:354–363. <https://doi.org/10.1165/rcmb.2018-0278OC>
- Honda JR, Knight V, Chan ED (2015) Pathogenesis and risk factors for nontuberculous mycobacterial lung disease. Clin Chest Med 36:1–11. <https://doi.org/10.1016/j.ccm.2014.10.001>
- Horne D, Skerrett S (2019) Recent advances in nontuberculous mycobacterial lung infections. F1000Res 8:F1000. <https://doi.org/10.12688/f1000research.20096.1>

- Hoy SM (2021) Amikacin liposome inhalation suspension in refractory *Mycobacterium avium* complex lung disease: A profile of its use. Clin Drug Investig 41:405–412. <https://doi.org/10.1007/s40261-021-01010-z>
- Huang HL, Lu PL, Lee CH et al. (2020) Treatment of pulmonary disease caused by *Mycobacterium kansasii*. J Formos Med Assoc 119(Suppl. 1):S51–S57. <https://doi.org/10.1016/j.jfma.2020.05.018>
- Jamal F, Hammer MH (2022) Nontuberculous mycobacterial infections. Radiol Clin North Am 60:399–408. <https://doi.org/10.1016/j.rcl.2022.01.012>
- Johnson MM, Odell JA (2014) Nontuberculous mycobacterial pulmonary infections. J Thorac Dis 6:210–220. <https://doi.org/10.3978/j.issn.2072-1439.2013.12.24>
- Johnston JC, Chiang L, Elwood K (2014) *Mycobacterium kansasii*. Microbiol Spectr 5. J Thorac Dis 6(3):210–220. <https://doi.org/10.3978/j.issn.2072-1439.2013.12.24>
- Kambali S, Quinonez E, Sharifi A et al. (2021) Pulmonary nontuberculous mycobacterial disease in Florida and association with large-scale natural disasters. BMC Public Health 21:2058. <https://doi.org/10.1186/s12889-021-12115-7>
- Kim BG, Jhun BW, Kim H et al. (2022) Treatment outcomes of *Mycobacterium avium* complex pulmonary disease according to disease severity. Sci Rep 12:1970. <https://doi.org/10.1038/s41598-022-06022-z>
- Kim BJ, Hong SH, Yu HK et al. (2013) *Mycobacterium parakoreense* sp. nov., a slowly growing non-chromogenic species related to *Mycobacterium koreense*, isolated from a human clinical specimen. Int J Syst Evol Microbiol 63:2301–2308. <https://doi.org/10.1099/ijs.0.045070-0>
- Kim JY, Lee HW, Yim JJ et al. (2023) Outcomes of adjunctive surgery in patients with nontuberculous mycobacterial pulmonary disease: A systematic review and meta-analysis. Chest 163:763–777. <https://doi.org/10.1016/j.chest.2022.09.037>
- Kim JY, Park S, Park IK et al. (2021) Outcomes of adjunctive surgery for nontuberculous mycobacterial pulmonary disease. BMC Pulm Med 21:312. <https://doi.org/10.1186/s12890-021-01679-0>
- Koh WJ (2017) Nontuberculous mycobacteria-overview. Microbiol Spectr 5(1):TNMI7-0024-2016. <https://doi.org/10.1128/microbiol-spec.tnmi7-0024-2016>
- Koh WJ, Moon SM, Kim SY et al. (2017) Outcomes of *Mycobacterium avium* complex lung disease based on clinical phenotype. Eur Respir J 50:1602503. <https://doi.org/10.1183/13993003.02503-2016>
- Kumar K, Daley CL, Griffith DE et al. (2022) Management of *Mycobacterium avium* complex and *Mycobacterium abscessus* pulmonary disease: Therapeutic advances and emerging treatments. Eur Respir Rev 31:210212. <https://doi.org/10.1183/16000617.0212-2021>
- Kumar K, Ponnuswamy A, Capstick TG et al. (2024) Non-tuberculous mycobacterial pulmonary disease (NTM-PD): Epidemiology, diagnosis and multidisciplinary management. Clin Med 24:100017. <https://doi.org/10.1016/j.clinme.2024.100017>
- Kwak N, Hwang HW, Kim HJ et al. (2022) The association between Bacille Calmette-Guérin vaccination and nontuberculous mycobacterial pulmonary disease. J Korean Med Sci 37:e206. <https://doi.org/10.3346/jkms.2022.37.e206>
- Larsson LO, Polverino E, Hoefsloot W et al. (2017) Pulmonary disease by non-tuberculous mycobacteria – Clinical management, unmet needs and future perspectives. Expert Rev Respir Med 11:977–989. <https://doi.org/10.1080/17476348.2017.1386563>
- Laudone TW, Garner L, Kam CW et al. (2021) Novel therapies for treatment of resistant and refractory nontuberculous mycobacterial infections in patients with cystic fibrosis. Pediatr Pulmonol 56(Suppl. 1):S55–S68. <https://doi.org/10.1002/ppul.24939>
- Lee JY, Choi EH (2022) Skin infection caused by *Mycobacterium abscessus* in a healthy adult. J Mycol Infect 27:38–40. <https://doi.org/10.17966/JMI.2022.27.2.38>
- Li J, Zhan L, Qin C (2021) The double-sided effects of *Mycobacterium bovis* bacillus Calmette-Guérin vaccine. NPJ Vaccines 6:14. <https://doi.org/10.1038/s41541-020-00278-0>
- Loebinger MR (2017) *Mycobacterium avium* complex infection: Phenotypes and outcomes. Eur Respir J 50:1701380. <https://doi.org/10.1183/13993003.01380-2017>
- Loebinger MR, Quint JK, van der Laan R et al. (2023) Risk factors for nontuberculous mycobacterial pulmonary disease: A systematic literature review and meta-analysis. Chest 164:1115–1124. <https://doi.org/10.1016/j.chest.2023.06.014>
- Lopeman RC, Harrison J, Desai M et al. (2019) *Mycobacterium abscessus*: Environmental bacterium turned clinical nightmare. Microorganisms 7:90. <https://doi.org/10.3390/microorganisms7030090>
- Lu M, Fitzgerald D, Karpelowsky J et al. (2018) Surgery in nontuberculous mycobacteria pulmonary disease. Breathe (Sheff) 14:288–301. <https://doi.org/10.1183/20734735.027218>
- Meliefste HM, Mudde SE, Ammerman NC et al. (2024) A laboratory perspective on *Mycobacterium abscessus* biofilm culture, characterization and drug activity testing. Front Microbiol 15:1392606. <https://doi.org/10.3389/fmicb.2024.1392606>
- Mencarini J, Cresci C, Simonetti MT et al. (2017) Non-tuberculous mycobacteria: Epidemiological pattern in a reference laboratory and risk factors associated with pulmonary disease. Epidemiol Infect 145(3):515–522. <https://doi.org/10.1017/S0950268816002521>
- Mercaldo RA, Marshall JE, Cangelosi GA et al. (2023) Environmental risk of nontuberculous mycobacterial infection: Strategies for advancing methodology. Tuberculosis 139:102305. <https://doi.org/10.1016/j.tube.2023.102305>
- Moore M, Frerichs JB (1953) An unusual acid-fast infection of the knee with subcutaneous, abscess-like lesions of the gluteal region. J Invest Dermatol 20:133–169. <https://doi.org/10.1038/jid.1953.18>
- Moral MZ, Desai K, Arain AR et al. (2019) *Mycobacterium abscessus*-associated vertebral osteomyelitis in an immunocompetent patient: A rare case report and literature review. Spinal Cord Ser Cases 5:53. <https://doi.org/10.1038/s41394-019-0197-5>

- Morimoto K, Iwai K, Uchimura K et al. (2014) A steady increase in nontuberculous mycobacteriosis mortality and estimated prevalence in Japan. *Ann Am Thorac Soc* 11:1–8. <https://doi.org/10.1513/AnnalsATS.201303-067OC>
- Morimoto K, Nonaka M, Yamazaki Y et al. (2024) Amikacin liposome inhalation suspension for *Mycobacterium avium* complex pulmonary disease: A subgroup analysis of Japanese patients in the randomized, phase 3, CONVERT study. *Respir Investig* 62:284–290. <https://doi.org/10.1016/j.resinv.2023.12.012>
- Nair VR, Franco LH, Zacharia VM et al. (2016) Microfold cells actively translocate *Mycobacterium tuberculosis* to initiate infection. *Cell Rep* 16:1253–1258. <https://doi.org/10.1016/j.celrep.2016.06.080>
- Natanti A, Palpacelli M, Valsecchi M et al. (2021) *Mycobacterium chimaera*: A report of 2 new cases and literature review. *Int J Legal Med* 135:2667–2679. <https://doi.org/10.1007/s00414-021-02630-y>
- Ndlovu H, Marakalala MJ (2016) Granulomas and inflammation: Host-directed therapies for tuberculosis. *Front Immunol* 7:434. <https://doi.org/10.3389/fimmu.2016.00434>
- Nie W, Duan H, Huang H et al. (2014) Species identification of *Mycobacterium abscessus* subsp. *abscessus* and *Mycobacterium abscessus* subsp. *bolletii* using rpoB and hsp65, and susceptibility testing to eight antibiotics. *Int J Infect Dis* 25:170–174. <https://doi.org/10.1016/j.ijid.2014.02.014>
- Orujyan D, Narinyan W, Rangarajan S et al. (2022) Protective efficacy of BCG vaccine against *Mycobacterium leprae* and non-tuberculous mycobacterial infections. *Vaccines (Basel)* 10:390. <https://doi.org/10.3390/vaccines10030390>
- Park HE, Lee W, Choi S et al. (2022) Modulating macrophage function to reinforce host innate resistance against *Mycobacterium avium* complex infection. *Front Immunol* 13:931876. <https://doi.org/10.3389/fimmu.2022.931876>
- Parte AC (2014) LPSN – list of prokaryotic names with standing in nomenclature. *Nucleic Acids Res* 42(Database issue): D613–D616. <https://doi.org/10.1093/nar/gkt111>
- Pathak K, Hart S, Lande L (2022) Nontuberculous mycobacteria lung disease (NTM-LD): Current recommendations on diagnosis, treatment, and patient management. *Int J Gen Med* 15: 7619–7629. <https://doi.org/10.2147/IJGM.S272690>
- Pennington KM, Vu A, Challener D et al. (2021) Approach to the diagnosis and treatment of non-tuberculous mycobacterial disease. *J Clin Tuberc Other Mycobact Dis* 24:100244. <https://doi.org/10.1016/j.jctube.2021.100244>
- Pereira AC, Ramos B, Reis AC et al. (2020) Non-tuberculous mycobacteria: Molecular and physiological bases of virulence and adaptation to ecological niches. *Microorganisms* 8:1380. <https://doi.org/10.3390/microorganisms8091380>
- Pidot SJ, Porter JL, Lister T et al. (2021) In vitro activity of SPR719 against *Mycobacterium ulcerans*, *Mycobacterium marinum* and *Mycobacterium chimaera*. *PLoS Negl Trop Dis* 15:e0009636. <https://doi.org/10.1371/journal.pntd.0009636>
- Pinner M (1935) Atypical acid-fast microorganisms. III. Chromogenic acid-fast bacilli from human beings. *American Review of Tuberculosis* 32(4):424–439.
- Prevots DR, Marras TK (2015) Epidemiology of human pulmonary infection with nontuberculous mycobacteria: A review. *Clin Chest Med* 36:13–34. <https://doi.org/10.1016/j.ccm.2014.10.002>
- Prevots DR, Shaw PA, Strickland D et al. (2010) Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. *Am J Respir Crit Care Med* 182:970–976. <https://doi.org/10.1164/rccm.201002-0310OC>
- Quang NT, Jang J (2021) Current molecular therapeutic agents and drug candidates for *Mycobacterium abscessus*. *Front Pharmacol* 12:724725. <https://doi.org/10.3389/fphar.2021.724725>
- Ratnatunga CN, Lutzky VP, Kupz A et al. (2020) The rise of non-tuberculosis mycobacterial lung disease. *Front Immunol* 11:303. <https://doi.org/10.3389/fimmu.2020.00303>
- Riccardi N, Monticelli J, Antonello RM et al. (2020) *Mycobacterium chimaera* infections: An update. *J Infect Chemother* 26:199–205. <https://doi.org/10.1016/j.jiac.2019.11.004>
- Rodríguez-Temporal D, Herrera L, Alcaide F et al. (2023) Identification of *Mycobacterium abscessus* subspecies by MALDI-TOF mass spectrometry and machine learning. *J Clin Microbiol* 61:e0111022. <https://doi.org/10.1128/jcm.01110-22>
- Roth A, Reischl U, Schönfeld N et al. (2000) *Mycobacterium heckeshornense* sp. nov. A new pathogenic slowly growing *Mycobacterium* sp. causing cavitary lung disease in an immunocompetent patient. *J Clin Microbiol* 38:4102–4107. <https://doi.org/10.1128/JCM.38.11.4102-4107.2000>
- Ruis C, Bryant JM, Bell SC et al. (2021) Dissemination of *Mycobacterium abscessus* via global transmission networks. *Nat Microbiol* 6:1279–1288. <https://doi.org/10.1038/s41564-021-00963-3>
- Runyon EH (1959) Anonymous mycobacteria in pulmonary disease. *Med Clin North Am* 43:273–290. [https://doi.org/10.1016/s0025-7125\(16\)34193-1](https://doi.org/10.1016/s0025-7125(16)34193-1)
- Salvana EM, Cooper GS, Salata RA (2007) *Mycobacterium* other than tuberculosis (MOTT) infection: An emerging disease in infliximab-treated patients. *J Infect* 55:484–487. <https://doi.org/10.1016/j.jinf.2007.08.007>
- Schuurbiers MMF, Bruno M, Zweijpenning SMH et al. (2020) Immune defects in patients with pulmonary *Mycobacterium abscessus* disease without cystic fibrosis. *ERJ Open Res* 6:00590–2020. <https://doi.org/10.1183/23120541.00590-2020>
- Seth-Smith HMB, Imkamp F, Tagini F et al. (2019) Discovery and characterization of *Mycobacterium basiliense* sp. nov., a non-tuberculous *Mycobacterium* isolated from human lungs. *Front Microbiol* 9:3184. <https://doi.org/10.3389/fmicb.2018.03184>
- Shahraki AH, Trovato A, Mirsaedi M et al. (2017) *Mycobacterium persicum* sp. nov., a novel species closely related to *Mycobacterium kansasii* and *Mycobacterium gastri*. *Int J Syst Evol Microbiol* 67:1766–1770. <https://doi.org/10.1099/ijsem.0.001862>
- Sharma SK, Upadhyay V (2020) Epidemiology, diagnosis and treatment of non-tuberculous mycobacterial diseases. *Indian J Med Res* 152:185–226. [https://doi.org/10.4103/ijmr.IJMR\\_902\\_20](https://doi.org/10.4103/ijmr.IJMR_902_20)
- Shin MK, Shin SJ (2021) Genetic involvement of *Mycobacterium avium* complex in the regulation and manipulation of innate



- immune functions of host cells. *Int J Mol Sci* 22:3011. <https://doi.org/10.3390/ijms22063011>
- Shirley M (2019) Amikacin liposome inhalation suspension: A review in *Mycobacterium avium* complex lung disease. *Drugs* 79:555–562. <https://doi.org/10.1007/s40265-019-01095-z>
- Shu CC, Wu MF, Pan SW et al. (2020) Host immune response against environmental nontuberculous mycobacteria and the risk populations of nontuberculous mycobacterial lung disease. *J Formos Med Assoc* 119(Suppl. 1):S13–S22. <https://doi.org/10.1016/j.jfma.2020.05.001>
- Shulha JA, Escalante P, Wilson JW (2019) Pharmacotherapy approaches in nontuberculous mycobacteria infections. *Mayo Clin Proc* 94:1567–1581. <https://doi.org/10.1016/j.mayocp.2018.12.011>
- Sousa S, Borges V, Joao I et al. (2019) Nontuberculous mycobacteria persistence in a cell model mimicking alveolar macrophages. *Microorganisms* 7:113. <https://doi.org/10.3390/microorganisms7050113>
- Steglich R, Dalcolmo GF, Carvalho de Queiroz Mello F et al. (2020) Non-tuberculous mycobacteria: Epidemiological pattern in a reference laboratory and risk factors associated with pulmonary disease. *BMC Public Health* 20:1593.
- Tam CM, Leung CC (2000) Cessation of the BCG (Bacille Calmette Guérin) revaccination programme for primary school children in Hong Kong. *Public Health Epidemiol Bull* 9:25–27.
- Taylor LJ, Mitchell JD (2023) Surgical resection in nontuberculous mycobacterial pulmonary disease. *Clin Chest Med* 44:861–868. <https://doi.org/10.1016/j.ccm.2023.06.013>
- Thomson RM, Donnan E, Konstantinos A (2017) Notification of nontuberculous mycobacteria: An Australian perspective. *Ann Am Thorac Soc* 14:318–323. <https://doi.org/10.1513/AnnalsATS.201612-994OI>
- Thomson RM, Furuya-Kanamori L, Coffey C et al. (2020) Influence of climate variables on the rising incidence of nontuberculous mycobacterial (NTM) infections in Queensland, Australia 2001–2016. *Sci Total Environ* 740:139796. <https://doi.org/10.1016/j.scitotenv.2020.139796>
- Thomson RM, Loebinger MR, Burke AJ et al. (2023) OPTIMA: An open-label, non-comparative pilot trial of inhaled molgramostim in pulmonary nontuberculous mycobacterial infection. *Ann Am Thorac Soc* 21:568–576. <https://doi.org/10.1513/AnnalsATS.202306-532OC>
- Thornton CS, Mellett M, Jarand J et al. (2021) The respiratory microbiome and nontuberculous mycobacteria: An emerging concern in human health. *Eur Respir Rev* 30:200299. <https://doi.org/10.1183/16000617.0299-2020>
- Torrelles JB, Schlesinger LS (2017) Integrating lung physiology, immunology, and tuberculosis. *Trends Microbiol* 25:688–697. <https://doi.org/10.1016/j.tim.2017.03.007>
- Tortoli E (2014) Microbiological features and clinical relevance of new species of the genus *Mycobacterium*. *Clin Microbiol Rev* 27:727–752. <https://doi.org/10.1128/CMR.00035-14>
- Tortoli E, Fedrizzi T, Meehan CJ et al. (2017) The new phylogeny of the genus *Mycobacterium*: The old and the news. *Infect Genet Evol* 56:19–25. <https://doi.org/10.1016/j.meegid.2017.10.013>
- Tortoli E, Rindi L, Garcia MJ et al. (2004) Proposal to elevate the genetic variant MAC-A, included in the *Mycobacterium avium* complex, to species rank as *Mycobacterium chimaera* sp. nov. *Int J Syst Evol Microbiol* 54:1277–1285. <https://doi.org/10.1099/ijs.0.02777-0>
- van der Laan R, Snablić A, Obradovic M (2022) Meeting the challenges of NTM-PD from the perspective of the organism and the disease process: Innovations in drug development and delivery. *Respir Res* 23:376. <https://doi.org/10.1186/s12931-022-02299-w>
- Varma-Basil M, Bose M (2019) Mapping the footprints of nontuberculous mycobacteria: A diagnostic dilemma. In: Velayati AA, Farnia P (eds) *Nontuberculous mycobacteria (NTM)*. London, Academic Press, pp. 155–175. eBook ISBN: 9780128146934.
- Vega-Dominguez P, Peterson E, Pan M et al. (2020) Biofilms of the non-tuberculous *Mycobacterium chelonae* form an extracellular matrix and display distinct expression patterns. *Cell Surf* 6:100043. <https://doi.org/10.1016/j.tcs.2020.100043>
- Verma D, Chan ED, Ordway DJ (2020) Non-tuberculous mycobacteria interference with BCG-current controversies and future directions. *Vaccines (Basel)* 8:688. <https://doi.org/10.3390/vaccines8040688>
- Victoria L, Gupta A, Gómez JL et al. (2021) *Mycobacterium abscessus* complex: A review of recent developments in an emerging pathogen. *Front Cell Infect Microbiol* 11:659997. <https://doi.org/10.3389/fcimb.2021.659997>
- Watanabe C, Yoshida Y, Kidoguchi G et al. (2023) Disseminated *Mycobacterium abscessus* infection with osteoarticular manifestations as an important differential diagnosis of inflammatory arthritis: A case report and literature review. *Mod Rheumatol Case Rep* 8:49–54. <https://doi.org/10.1093/mrcr/rxad054>
- Waugh KM, Wajahat R (2023) Pulmonary *Mycobacterium abscessus* infection: A pathogen in disguise. *Cureus* 15:e46897. <https://doi.org/10.7759/cureus.46897>
- Weeratunga P, Moller DR, Ho LP (2024) Immune mechanisms of granuloma formation in sarcoidosis and tuberculosis. *J Clin Invest* 134:e175264. <https://doi.org/10.1172/JCI175264>
- Wilińska E, Szturmowicz M (2010) [Lung mycobacteriosis – clinical presentation, diagnostics and treatment]. *Pneumonol Alergol Pol* 78:138–147.
- Winthrop KL, Flume PA, Thomson R et al. (2021) Amikacin liposome inhalation suspension for *Mycobacterium avium* complex lung disease: A 12-month open-label extension clinical trial. *Ann Am Thorac Soc* 18:1147–1157. <https://doi.org/10.1513/AnnalsATS.202008-925OC>
- Yoo SJ, Lee KH, Jung SN et al. (2013) Facial skin and soft tissue infection caused by *Mycobacterium wolinskyi* associated with cosmetic procedures. *BMC Infect Dis* 13:479. <https://doi.org/10.1186/1471-2334-13-479>