



Universiteit
Leiden
The Netherlands

Advancing host-directed therapy for *Mycobacterium avium* infection: identification of drug candidates and potential host targets

Kilinc, G.

Citation

Kilinc, G. (2025, November 6). *Advancing host-directed therapy for Mycobacterium avium infection: identification of drug candidates and potential host targets*. Retrieved from <https://hdl.handle.net/1887/4282119>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/4282119>

Note: To cite this publication please use the final published version (if applicable).



Chapter

1

General introduction

Gül Kiling¹

¹ Leiden University Center for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

Background & milestones NTM research

Tuberculosis (TB) is an ancient disease characterized by the presence of tubercles in tissues like the lungs and therefore historically described to be caused by “tubercle bacilli”. In 1882, Robert Koch isolated and identified the causative pathogen of TB and renamed it *Mycobacterium tuberculosis* (*Mtb*), which is the main cause of human infections due to *Mycobacterium* species (1). Following Koch’s discovery, other species of *Mycobacterium* were increasingly identified, which were referred to by several names, including ‘atypical mycobacteria’ and ‘nontuberculous mycobacteria’ (NTM). The earliest report of NTM was in the late 1880s when Alvarez and Tavit described the smegma bacillus (currently known as *Mycobacterium smegmatis*) found in human secretions (2). Nonetheless, it was already in 1868 in England, when Crisp observed seemingly TB in chicken (avian), later classified as *Mycobacterium tuberculosis avium* that mimicked the disease seen in humans, which was the first probable description of a bacterium now known as *Mycobacterium avium* (*Mav*) (**Figure 1**) (3). Koch initially stated that *Mav* was rather a variant of *Mtb* in animals, but more and more evidence became available to counteract his argument (3). According to Maffucci’s reports in 1890 and 1892, Rivolta suggested in 1883 and eventually also showed by experimental methods in 1889 that there was a difference between bovine TB and *Mav* found in chickens. In his reports, Maffucci described that *Mav* was definitely distinct from *Mtb* in the sense of cultural and pathogenic aspects, which was also confirmed by Cadiot, Gilbert, and Roger. However, since guinea pigs injected with *Mav* did not develop disease, *Mav* was believed not to cause disease in humans (3, 4). The development of improved culture techniques resulted in more accurate diagnoses of mycobacterial disease. In 1933, Branch reported the recognition of human-derived (pathogenic) *Mav* strains, and in 1943 Feldman et al. described a virulent *Mav* strain isolated from a patient with lung disease (5, 6). In 1949, a report by Cuttino and McCabe described a case of disseminated disease caused by a bacterial species, which was first named *Nocardia intracellularis*, later renamed to *Mycobacterium intracellulare* (*Min*) (7). Since *Mav* and *Min* are genetically very similar and not distinguishable by common laboratory examinations, they were together referred to as the *Mav* complex (MAC) (8). By 1953, more cases of MAC were described (9), and MAC was considered the most common cause of chronic lung infection due to NTM worldwide in the 1970s, which is still the case in many geographical regions. Interest in NTM increased in 1982, when disseminated infection, particularly caused by *Mav*, was dramatically more often observed in human immunodeficiency virus (HIV)-infected patients. While initially extremely rare, the recognition of *Mav* in patients with acquired immunodeficiency syndrome (AIDS) increased the number of disseminated cases strongly (10). Initially treated with solely *Mtb*-specific drugs, the implementation of clarithromycin in the 1990s marked a significant breakthrough in managing MAC disease. Meanwhile, the occurrence of MAC infections in AIDS patients was the first indication of the current knowledge that host immunity, specifically cell-mediated immunity, is critical for protection against MAC.

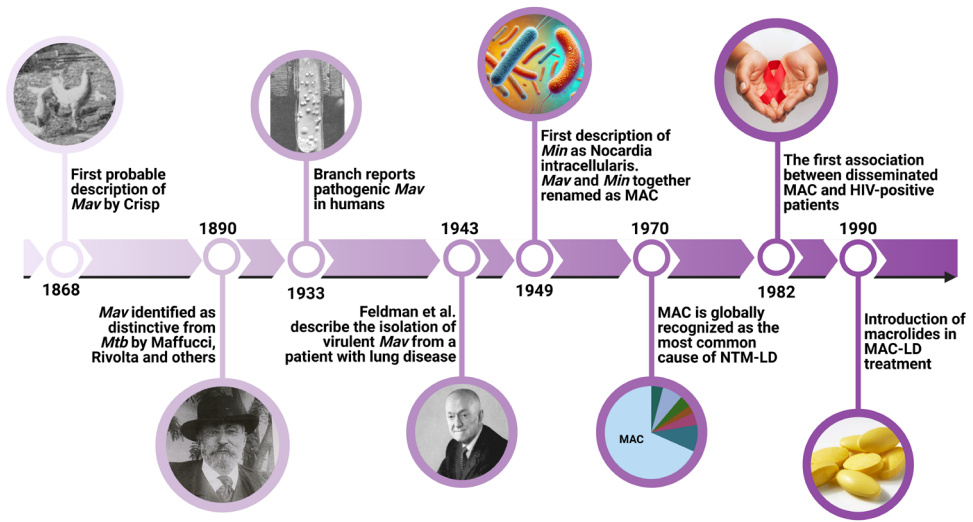


Figure 1. History of *Mav* (complex). *Mav*: *Mycobacterium avium*, *Mtb*: *Mycobacterium tuberculosis*, *Min*: *Mycobacterium intracellulare*, MAC: *Mycobacterium avium* complex, NTM: nontuberculous mycobacteria, LD: lung disease, HIV: human immunodeficiency virus. Created with BioRender.

MAC pathogenesis

Entry in, and recognition by host cells of the immune system

Given the airway-oriented nature of NTM infections, *Mav* may invade the mucosal barrier by interacting with bronchial epithelial cells to cause infection (11). Recognition and uptake of *Mav* by immune cells begins with the interaction of pattern recognition receptors (PRRs) on the cell surface that bind to pathogen-associated molecular patterns (PAMPs) to initiate a protective innate immune response against the mycobacteria. Characterization of the adhesions on *Mav* cell surface associated with the ability to interact with epithelial cells has identified the bacterial fibronectin attachment protein (FAP). FAP interacts with fibronectin to bind to integrin receptors on the surface of bronchial epithelial cells (12, 13). Once *Mav* reaches the alveolar space, it interacts with alveolar epithelial cells. Once recognized, *Mav* is taken up by epithelial cells requiring structural modifications of the cytoskeleton and proactive engagement of the cell (14). It is believed that *Mav*, by inducing biofilm formation and impairing the induction of an inflammatory response, may establish a chronic lung infection using the alveolar epithelial cells as a niche (15-17). While the mechanisms of escaping epithelial cells are unknown, it has been shown that *Mav* leaving epithelial mucosa has a different phenotype resulting in more efficient invasion of macrophages (18).

The mycobacteria may also directly, without interaction with epithelial cells, reach mononuclear phagocytes like monocytes and macrophages in the airways (19). There is a general consensus that macrophages represent the main reservoir of mycobacteria in the host (20, 21). Macrophages have a wide range of activation states with different functions, which can be broadly classified into two polar ends of the activation spectrum: pro-inflammatory macrophages (M1), involved in fighting infections, and

anti-inflammatory macrophages (M2), which play a role in resolving inflammation and promoting tissue repair (22-24). In the healthy “resting” state, human alveolar macrophages may possess an M1 or M2 phenotype (25, 26). During bacterial infections, however, host responses are skewing toward an M1 signature, which is associated with the control of acute infections. In contrast, the persistence of bacterial pathogens is linked to macrophage reprogramming to the M2 signature (27).

Macrophages express a wide variety of PRRs (28, 29), including Fc receptors, integrins, complement receptors (CR), C-type lectins, mannose and scavenger receptors. In addition to recognition, toll-like receptors (TLRs) are also involved in the induction of intracellular signaling cascades and pro-inflammatory responses. In particular TLR2, potentially by forming heterodimers with TLR1 and TLR6, plays a pivotal role in innate immune protection against *Mav* infection (30, 31). NTM, including *Mav*, express glycopeptidolipids (32), a major cell surface component that shields cell wall phosphatidyl-myo-inositol mannosides, thereby weakening recognition by TLR2 (33). Moreover, TLR6 and TLR9 are indispensable for managing *Mav* infection in mice (34, 35).

Host-pathogen interactions: macrophages vs. *Mav*

Once *Mav* is recognized, the macrophage membrane encapsulates and phagocytoses the mycobacteria, causing *Mav* to be targeted to cytoplasmic vacuoles called phagosomes. These phagosomes engage with the endosomal compartment to promote phagosome maturation (36). Phagosomes ultimately fuse with lysosomes that contain enzymes for bacterial killing (**Figure 2**). However, MAC can prevent its killing for example by impairing phagosome maturation by, using its secretory protein MAV_2941, interfering with vesicle trafficking and consequently fusion with lysosomes (37, 38). Moreover, mycobacterial membrane protein large 4 (MMPL4) participates in preventing phagosome maturation in *Mav*-infected cells by mechanisms not yet understood (39). Mycobacteria like *Mtb* and *Mycobacterium marinum* (*Mmar*) are known to be able to escape from the phagosome into the cytosol (40), where they can be targeted to autophagosomes to be degraded in a process called autophagy (or xenophagy) (41). In the same study, *Mav* remained phagosomal and showed no translocation to the cytosol, but the possibility of phagosomal escape has not been conclusively disproven.

In addition to direct recognition of *Mav*, macrophages can further be activated by IFN- γ released by CD4⁺ T helper 1 (Th1) cells induced by dendritic cells (DCs) via amongst others IL-12 (42). By presenting antigens and inducing T-cell responses, DCs link innate and adaptive immunity (43), in which the CD4⁺ T cell subset is essential for the host immunity against *Mav* (44, 45). Activation of the macrophage results in the TLR2-mediated production of pro-inflammatory cytokines, like IL-12, IL-23, and TNF (46, 47). IL-12 and IL-23 secreted by macrophages bind to their receptors on Th1 cells, promoting an increase in IFN- γ production. Furthermore, TNF induces apoptosis upon binding to its receptor TNFR1 (48). While most research indicates (TNF-mediated) host cell apoptosis as a host defense mechanism against mycobacterial, including *Mav*, infection (49-52), apoptosis can also be considered as a virulence mechanism of the bacteria as apoptotic macrophages have also been shown to result in the release and dissemination of *Mav* infection (53, 54). *Mav* expresses the MAV_2054 protein, which is known to induce macrophage apoptosis that can therefore be either host-protective or host-detrimental during *Mav* infection (55). Finally, macrophages generate reactive

oxygen species (ROS) and reactive nitrogen species (RNS) upon activation. While *Mav* tolerates RNS (56), ROS has been described to be involved in the killing of *Mav* by macrophages (57, 58). Taken together, while macrophages are the first-line defenders against *Mav* infection, bacteria can modulate host immune function to establish an intracellular replication niche that facilitates their replication and survival and evades immune detection.

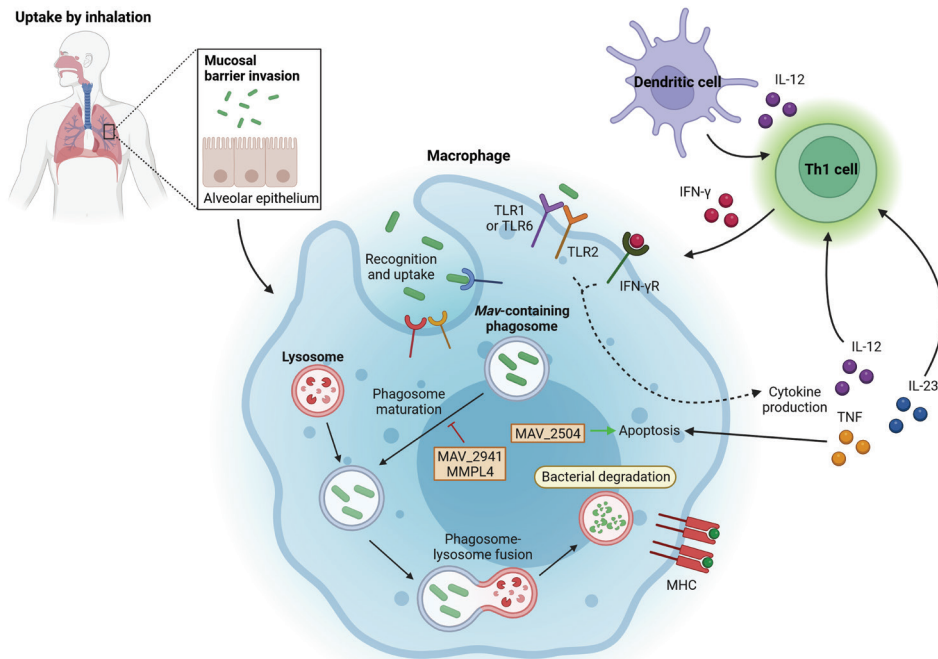


Figure 2. Phagocytosis and elimination of *Mav* by alveolar macrophages. Created with BioRender.

***Mav* exposure and risk factors**

Environmental factors

While there is some evidence of human-to-human transmission (59), this type of transmission is extremely rare. Reasons may be the opportunistic nature of *Mav*, limiting infection in healthy individuals, and for example the lack of human-specific adaptations required for widespread transmission. Hence, it is believed that human disease due to *Mav* is acquired from environmental exposures. *Mav* and other NTM have been isolated from various environmental habitats, including both natural and treated water sources (e.g. drinking water distribution systems, hospitals, and household plumbing) (**Figure 3**), which are shared with humans and animals and have been associated with *Mav* disease (60-63). In addition to water, bacteria aerosolized as dust from potting soil has also been shown to be a risk factor for the development of disease due to *Mav* (64, 65). While the isolation of NTM from the environment is similar among different geographic areas (66, 67), higher risks for NTM infection and disease were identified in areas

characterized by higher population densities and higher household education and income levels. These factors tend to cluster in more urbanized areas, which previously have been linked to NTM disease (68-70).

The major factor that permits the persistence of *Mav* and other NTM in environmental sources is their hydrophobic, lipid-rich outer membrane (71, 72). The hydrophobic characteristic of these bacteria enables their attachment to surfaces (73), which prevents bacteria from being washed out and allows them to form biofilms (74). Both the character of a thick cell wall and biofilm formation result in the increased tolerance of NTM to antibiotics and disinfectants.

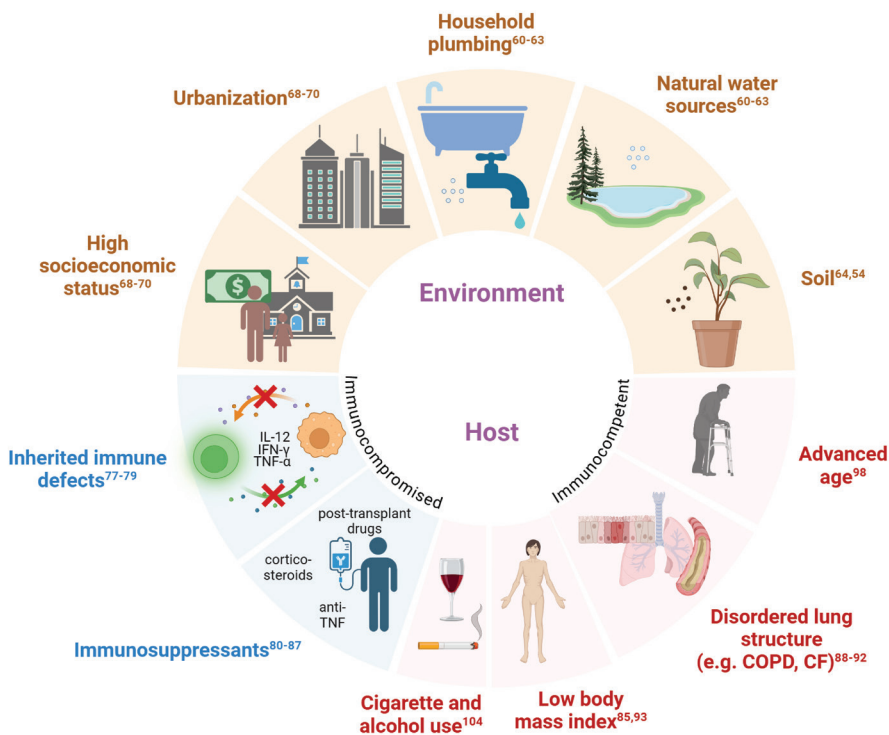


Figure 3. Environmental and host risk factors for MAC-LD. Created with BioRender.

Host factors

Due to their abundance, nearly everyone is presumed to be exposed to NTM, including *Mav*. Nevertheless, most people do not develop clinical signs or disease, indicating that host factors must also be involved in the outcome of exposure and infection. This was first reflected by the well-established association between disseminated NTM infections, particularly by *Mav*, in AIDS patients (75, 76), while the incidence of disseminated disease in this group was reduced by the administration of antiretroviral therapy. The key role of host immunity in the outcome of *Mav* infection is further supported by the development of lung disease (*Mav*-LD) in other immunocompromised phenotypes.

Inherited defects in the IFN- γ /IL-12 signaling pathways are known to be associated with increased susceptibility to mycobacterial infection and diseases, including *Mav* (77-79) (**Figure 3**), indicating that IFN- γ and IL-12 are both crucial elements in the host defense against NTM. Another pro-inflammatory cytokine induced upon *Mav* infection is TNF and its important role in controlling intracellular mycobacteria is shown by anti-TNF therapy; in several autoimmune diseases, targeting the TNF pathway with anti-TNF therapies, such as infliximab, adalimumab, and etanercept, increases the risk of the development of active TB (80), but also of *Mav* disease (81, 82). Similar to subjects receiving TNF blockers, patients receiving immunosuppressive drugs like corticosteroids (83-85), but also medication (e.g. tacrolimus) provided following organ transplantation (86), have higher rates of *Mav*-LD. Furthermore, individuals with solid tumors are at an elevated risk of developing lung disease caused by NTM, likely due to immune dysfunction associated with the disease or the immunosuppressive effects of chemotherapy (87).

However, *Mav* infections can also occur in hosts who are apparently healthy, without systemic immunosuppression, but often have (pre-existing) lung diseases or specific host characteristics (88). For example, cystic fibrosis (CF), an inherited disorder caused by mutations in the *CFTR* gene, leads to a reduced mucus layer and impaired mucociliary clearance, heightening the risk of the establishment of bacterial infection (89). Similarly, individuals with chronic obstructive pulmonary disease (COPD) or a history of pulmonary TB often have damaged lung structures, associated with a higher occurrence of *Mav*-LD (86, 90-92). Furthermore, lower body fat mass and BMI correlate with faster progression of *Mav*-LD (85, 93), which may be explained by the higher adiponectin and lower leptin levels expressed by fat cells, which have immunomodulatory effects (94-97). Furthermore, aging also increases susceptibility to *Mav* infection (98). This may be due to the simple fact that predisposing factors for *Mav* infection are more common with aging. However, independent of these underlying predisposing conditions, aging is also associated with immunosenescence that can affect key host defenses (99). With regards to gender, middle-aged (post-menopausal) females have a higher risk for *Mav*-LD (100-102), which may be related to the lower levels of estrogen as this has been shown to enhance the clearance of MAC in mice (103), although human data remain inconclusive (86, 87). In addition, middle-aged males with a history of smoking, alcohol use or aforementioned underlying lung diseases also have an increased risk for *Mav*-LD (104).

Although associations with some predisposing conditions are noticeably clear, predicting which individuals will develop *Mav* disease is not feasible. Nevertheless, factors that affect the host's susceptibility to MAC infection have enhanced our understanding of the pathogenesis of MAC, underscoring the significant role of the host's immune system in MAC infection.

Clinical presentations of *Mav* infection

Overall, *Mav* disease displays a range of clinical manifestations, from localized to systemic disease, largely influenced by the host's immune status and underlying risk factors. The most common site of *Mav* disease is the lung. *Mav*-LD can have two distinct forms (105, 106). Fibrocavitary lung disease, traditionally recognized as TB lung disease, is the severe form of *Mav*-LD and is characterized by areas of cavitation,

pleural thickening, volume loss, and fibrosis, mostly in the upper lobes of the lung. This form is more commonly seen in middle-aged males. Without appropriate treatment, fibrocavitary disease progresses within a few years and can result in respiratory failure or destruction (107, 108). Alternatively, *Mav*-LD can present as nodular-bronchiectatic disease, which is more commonly observed in slender and middle-aged women, affecting mainly the middle lobe of the lung with small nodules and bronchiectasis (109). Although this form has a much slower progression rate, long-term follow-up is nevertheless warranted, as progression still may lead to death.

Another manifestation of *Mav* is disseminated disease (106), which develops upon infection via inhalation or ingestion (gastrointestinal route), and mainly occurs in severely immunocompromised (CD4⁺ T cells counts < 100/uL) AIDS patients (45). Presently, the occurrence of disseminated *Mav* disease in AIDS patients has become rare due to effective antiviral therapies (110), however, disseminated disease remains life-threatening if untreated (19). Treatment of *Mav* in these cases is often considered lifelong unless immune function is restored.

Furthermore, *Mav* infection in children frequently presents as lymphadenitis, most likely acquired via ingestion and which primarily affects the cervical lymph nodes. Since antibiotics are typically less effective, excision by surgery, with generally high success rates, is the treatment of choice (104, 106).

Finally, while mainly caused by rapidly growing NTM like *Mycobacterium fortuitum* and *Mycobacterium abscessus*, *Mav* can also cause localized infections involving the skin, soft tissues, or bones, often developed upon exposure to contaminated water, trauma, or surgical wounds (111). Diagnosis and treatment are often hindered due to the failure to recognize rare organisms as the cause of infection and the infrequent routine performance of mycobacterial cultures for surgical wound infections. Once diagnosed, patients frequently receive both drugs and undergo excisional surgery.

Challenges in the management of *Mav*

Diagnosis and epidemiology of *Mav*-LD

Based on the 2007 guidelines from the American Thoracic Society and Infectious Diseases Society of America (ATS/IDSA), the diagnosis of *Mav*-LD necessitates compatible clinical symptoms, compatible radiographic findings, and repeated microbiological detection of the species (104). The symptoms of *Mav*-LD, however, can be variable and non-specific such as chronic and recurring cough, and may also include weight loss, fever, chest pain, or fatigue. Since such symptoms usually overlap with underlying lung diseases mentioned above, it is often difficult to recognize them as symptoms of *Mav*-LD. Hence, it is essential to exclude other diseases such as TB for which IFN- γ release assay may assist (112). The radiographic features of *Mav*-LD are dependent on whether it is fibrocavitary or nodular-bronchiectatic. Radiographic features can be assessed with a chest X-ray or, if cavitation is not observed, a chest high-resolution computer tomography (HRCT) scan. Since these physical and radiographic features are not sufficient to distinguish *Mav*-LD from other lung disorders like TB, microbiological confirmation is the third criterion for accurate diagnosis and treatment decisions. Identification of the causative pathogen can be achieved by molecular assays like 16S rRNA sequencing using a minimum of three sputum specimens

collected on separate days (113). In individuals who do not clearly meet the diagnostic criteria, a lung biopsy for diagnosing *Mav*-LD may be required (104). Diagnosing *Mav* infection requires the fulfillment of the equally important clinical, radiographic, and microbiologic criteria. Nevertheless, the diagnosis of *Mav* infection is often delayed due to non-specific symptoms, insufficient bacterial presence in sputum (114), resulting in late or incorrect treatment.

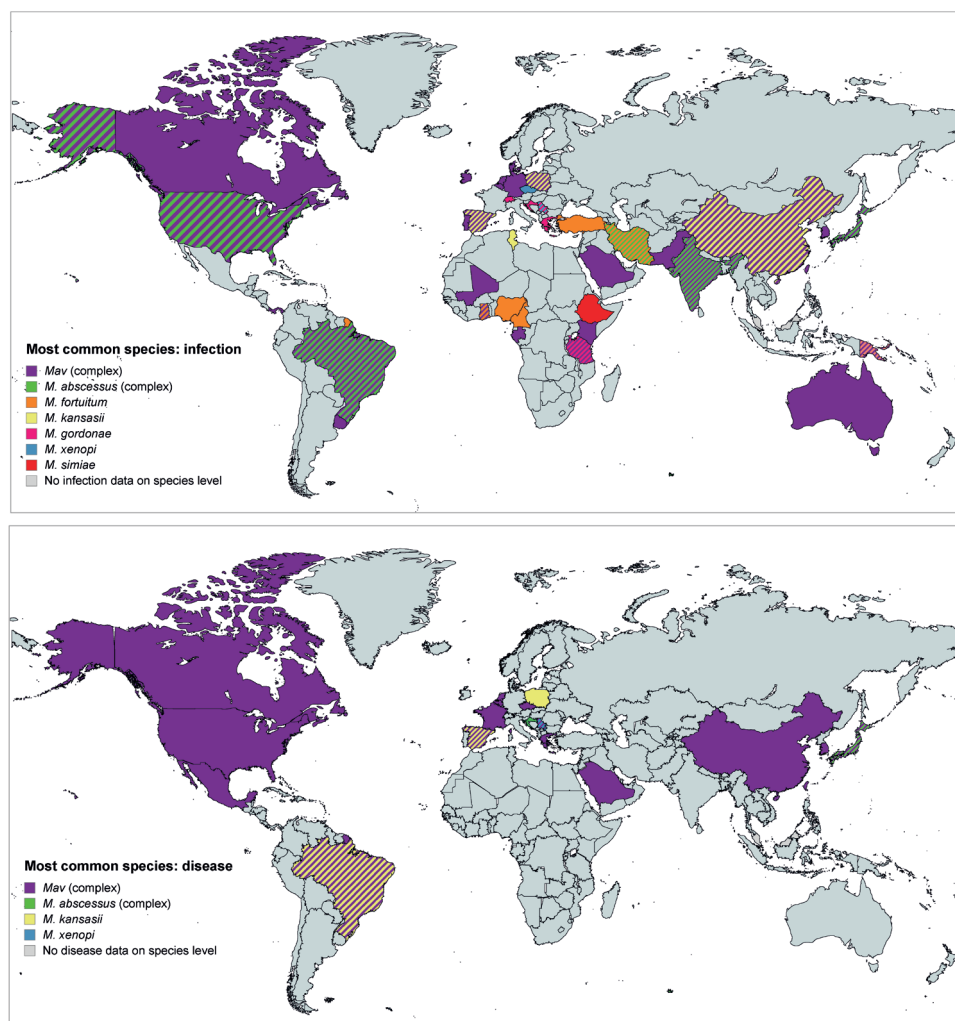


Figure 4. MAC is the most common species for NTM lung infection and disease worldwide. Created with MapChart.

Building on the challenges of diagnosing NTM, their reporting to public health authorities remains inconsistent. While NTM cases are seen in most industrialized countries, they are mandatorily reported in only a few states in the United States of America and Australia (115-117). The absence of a standardized global surveillance system limits the ability to accurately assess the burden of NTM to identify regional and national patterns

that would allow insight into potential individual or environmental risk factors for *Mav* infection and concomitant disease. Nevertheless, by using microbiological data from centralized public health institutions and administrative claims, a comprehensive review by Dahl et al. revealed that across numerous studies from more than 18 countries, the majority reported an overall increase in NTM lung infection (82%) and in lung disease (66.7-78%, depending on the case definition criteria used) (118). The most frequently isolated NTM was MAC with increased trends of infection and disease in 78.9% and 83.9% of the studies, respectively. While other NTM species can be more frequent in certain countries, MAC predominates for both NTM lung infection and disease (**Figure 4**) in most geographical regions (45, 119).

The overall increase in prevalence of *Mav* has likely multifactorial causes. Obvious reasons may be increased awareness or improved microbiologic detection techniques (105). Moreover, the occurrence of *Mav* cases may also increase as the aging population, associated with specific risk factors for *Mav* disease, as described above, is growing in certain countries such as the Netherlands (69, 120, 121). Finally, it has been suggested that by inducing protective immunity, TB infection provides cross-protection to NTM and the increasing number of diseases caused by NTM may be due to a decrease in TB cases (122). However, regardless of the reasons for the increase in NTM, the increase in the number of *Mav* infections and disease highlights the importance of documentation of NTM cases in a standardized manner to monitor and better manage these complicated infections.

Unsatisfactory treatment outcomes for *Mav*-LD

The recommended treatment of *Mav*-LD involves a combination of antibiotics, including a macrolide like clarithromycin or azithromycin, along with companion anti-TB drugs like ethambutol and a rifamycin to prevent the emergence of macrolide resistance (104). The goal of treatment is clinical improvement within 3-6 months and negative sputum cultures for 12 months while on therapy (123). However, the extensive and intensive nature of these antibiotic regimens may also hamper treatment adherence and increase the risk of developing drug resistance, complicating effective disease management. Despite a consensus statement in 2018 regarding treatment outcome definitions (124), there is a lack of widely accepted definitions of treatment success. The lack of such definitions, combined with different disease severities as well as different drug regimens and dosages included in MAC-LD clinical trials has resulted in inconsistent treatment success rates. Based on various systematic reviews and meta-analyses, Kwon et al. reported relatively poor pooled treatment success rates of 32-65% for MAC-LD (125). Successful treatment of MAC infection with a macrolide-based therapy is associated with the development of macrolide-resistant MAC strains (6.6-20% of treated patients) (126), for which the treatment regimens are far less successful (sputum conversion rates of 15-36%) (125). Even after initial success, 50% of the treated patients had a relapse (refractory infection) resulting in a positive sputum culture while receiving the same treatment (127, 128). Hence, the overall treatment success rate of the combinatorial antibiotic regime for MAC-LD has been unsatisfactory.

Several factors can interfere with successful treatment, which includes the lack of adherence to guidelines-based therapy (129), lack of treatment compliance or tolerance (77), lack of response to the regimen, the emergence of macrolide resistance, and

lack of effective treatment for macrolide-resistant disease (130-132). Moreover, MAC has also been associated with reinfection, which occurs in 25-48% of patients (133). Currently, only a small number of drugs, of which the majority have been repurposed, are evaluated in clinical trials for NTM-LD (134). This is likely a result of poor incentives, such as the lower profitability compared to communicable diseases like TB. Hence, new treatment strategies are urgently needed to potentiate, shorten and/or simplify current treatment strategies and improve treatment outcomes.

Antimicrobial susceptibility testing

For many years, antimicrobial susceptibility testing (AST) has long been conducted to predict the clinical effectiveness of antibiotics in treating NTM isolates. For MAC, the Clinical and Laboratory Standard Institute (CLSI) recommends using broth-based testing with both microdilution (multi-well plate) or macrodilution (radiometric BACTEC/Mycobacteria growth indicator tube (MGIT) system) (135, 136). However, unlike *Mtb* or rapidly growing mycobacteria (136, 137), it has long been known that for infections with slow growers like MAC, the correlation between *in vitro* susceptibility and good treatment outcomes for drugs is poor (138). Only *in vitro* susceptibility testing results for clarithromycin or clarithromycin-containing regimens correlated with *in vivo* efficacy (104), while the clinical response of MAC to ethambutol, rifampicin, and isoniazid using AST could not be predicted (139). Hence, the CLSI states that for MAC isolates only AST for clarithromycin is recommended.

The important discrepancies between AST results and the clinical response may stem from challenges in the laboratory process of AST, as well as the lack of standardized procedures and interpretation of the results. However, differential susceptibility *in vitro* versus *in vivo* may also be the result of specific bacterial behavior in a different setting. Suboptimal drug exposure and selection *in vivo* may differentially affect the interplay between tolerance and acquired resistance of bacteria interfering with susceptibility, which is not observed *in vitro* (140). Moreover, mycobacteria are known to be both intracellular and extracellular pathogens. Within cells, mycobacteria may adjust their metabolism or even become metabolically inactive (i.e. dormant) to prevent immune activation, possibly resulting in lower susceptibility to certain antibiotics that target active bacterial processes. Furthermore, *in vivo* bacteria might also reside in granulomas and biofilms which may affect their susceptibility to drugs. Hence, *in vitro* susceptibility testing of bacteria in conditions (more) resembling their physiological environment may improve the ability to translate the efficacy of drugs from *in vitro* to *in vivo*.

An alternative treatment strategy: boosting the host immune system

As reflected throughout this chapter, host immunity plays a crucial role in the outcome of *Mav* infection. Enhancing the host immune response to infection using host-directed therapy (HDT) may therefore be an alternative (adjunctive) treatment strategy to treat mycobacterial infections like *Mav*. HDT targets host processes to either reduce pathology caused by excessive inflammation or to enhance the host control of (intracellular) infection. The building knowledge on the host-pathogen interactions during *Mtb* infection has provided insights required for the development of HDT. By targeting host immunity rather than the pathogen, HDT has major advantages compared to conventional antibiotics including avoiding direct selective pressure on bacteria and

thus reducing the risk of *de novo* development of drug resistance, but also the potential to shorten the duration or decreasing the dosage of current treatment regimens, which may reduce adverse drug effects. Although HDT offers the potential to treat infections, the development of HDT for *Mav* is yet limited. The potential of HDT to boost the macrophage's ability to fight MAC infection has been shown *in vitro* with for example cytokines like GM-CSF or IFN- γ (141). Acquiring a more thorough understanding of how the host and pathogen interact during MAC infection may allow the development of other, more potent, HDTs.

Outline of this thesis

Given the challenges of current antibiotic treatments for *Mav*, this thesis focuses on developing HDTs to combat *Mav* infections. To this end, human cell-based infection models were developed to identify HDT candidates that improved host control of intracellular *Mav* infection. Using these models, also the host response to *Mav* infection was studied, improving our fundamental understanding of *Mav* infection and further aiding the development of HDTs against *Mav*.

First, we provide a comprehensive literature overview of HDTs under investigation for mycobacteria in **chapter 2**. As the development of HDTs for *Mav* is limited, this review mainly reports HDTs that have shown efficacy in treating *Mtb* infections. Moreover, we also propose potential intracellular host factors that may be targeted by HDT to improve host infection control of mycobacteria. In **chapter 3**, we developed human cell-based infection models for *Mav*, using the phagocytic MeJuSo cell line and primary human macrophages, to enable the identification of potential HDT candidates that can improve the antimycobacterial activity of host cells against intracellular *Mav*. These models can also be used to study host-pathogen interactions during *Mav* infection. By using the primary human macrophage model in **chapter 4** and **chapter 5**, we identified amiodarone and phenothiazines as potential HDT candidates for *Mav* infection. We showed that amiodarone most likely acts by enhancing the host autophagy pathway to impair intracellular survival of *Mav*, while phenothiazines impair intracellular *Mav* survival by enhancing cellular ROS production and additional mechanisms that remained undiscovered. In **chapter 6**, we performed transcriptomic analysis of primary human macrophages infected with *Mav* alongside *Mtb* to compare the host response between *Mav* and *Mtb* and to facilitate the rapid extrapolation of relevant findings from *Mtb* to *Mav*. The results described in **chapter 6** not only enhance our understanding of the host transcriptomic response to both pathogens, but they also provide insights into host factors that may be exploited for the development of HDT for *Mav*. Finally, the findings of this thesis were summarized and discussed in **chapter 7**.

References

1. Koch R. Classics in infectious diseases. The etiology of tuberculosis: Robert Koch. Berlin, Germany 1882. Rev Infect Dis. 1982;4(6):1270-4.
2. Wallace RJ, Jr., Nash DR, Tsukamura M, Blacklock ZM, Silcox VA. Human disease due to *Mycobacterium smegmatis*. J Infect Dis. 1988;158(1):52-9.
3. Feldman WH. Avian tuberculosis infections. Baltimore: The Williams & Wilkins company; 1938. 3 p. l., v-ix, 483 p. p.
4. Maffucci A. Die Hühnertuberculose. Zeitschrift für Hygiene und Infektionskrankheiten. 1892;11(1):445-86.
5. Branch A. A study of acid-fast organisms other than mammalian tubercle bacilli isolated from disease in man: The occurrence of avian tubercle bacillus infection of men and the diagnosis of avirulent

avian tubercle bacilli. *Tubercle*. 1933;14(8):337-53.

6. Feldman W, Davies R, Moses H, Andberg W. An Unusual *Mycobacterium* isolated from Sputum of a Man suffering from Pulmonary Disease of Long Duration. *American Review of Tuberculosis and Pulmonary Diseases*. 1943;48(2):82-93.

7. Cuttino JT, Mc CA. Pure granulomatous nocardiosis, a new fungus disease distinguished by intracellular parasitism; a description of a new disease in man due to a hitherto undescribed organism, *Nocardia intracellularis*, n. sp., including a study of the biologic and pathogenic properties of this species. *Am J Pathol*. 1949;25(1):1-47.

8. Wolinsky E. Nontuberculous mycobacteria and associated diseases. *Am Rev Respir Dis*. 1979;119(1):107-59.

9. Fogan L. Atypical mycobacteria. Their clinical, laboratory, and epidemiologic significance. *Medicine (Baltimore)*. 1970;49(3):243-55.

10. Horsburgh CR, Jr., Mason UG, 3rd, Farhi DC, Iseman MD. Disseminated infection with *Mycobacterium avium-intracellulare*. A report of 13 cases and a review of the literature. *Medicine (Baltimore)*. 1985;64(1):36-48.

11. Honda JR, Knight V, Chan ED. Pathogenesis and risk factors for nontuberculous mycobacterial lung disease. *Clin Chest Med*. 2015;36(1):1-11.

12. Schorey JS, Holsti MA, Ratliff TL, Allen PM, Brown EJ. Characterization of the fibronectin-attachment protein of *Mycobacterium avium* reveals a fibronectin-binding motif conserved among mycobacteria. *Mol Microbiol*. 1996;21(2):321-9.

13. Middleton AM, Chadwick MV, Nicholson AG, Dewar A, Groger RK, Brown EJ, et al. The role of *Mycobacterium avium* complex fibronectin attachment protein in adherence to the human respiratory mucosa. *Mol Microbiol*. 2000;38(2):381-91.

14. Bermudez LE, Young LS. Factors affecting invasion of HT-29 and HEp-2 epithelial cells by organisms of the *Mycobacterium avium* complex. *Infect Immun*. 1994;62(5):2021-6.

15. Bermudez LE, Goodman J. *Mycobacterium tuberculosis* invades and replicates within type II alveolar cells. *Infect Immun*. 1996;64(4):1400-6.

16. Carter G, Wu M, Drummond DC, Bermudez LE. Characterization of biofilm formation by clinical isolates of *Mycobacterium avium*. *J Med Microbiol*. 2003;52(Pt 9):747-52.

17. Li YJ, Danelishvili L, Wagner D, Petrofsky M, Bermudez LE. Identification of virulence determinants of *Mycobacterium avium* that impact on the ability to resist host killing mechanisms. *J Med Microbiol*. 2010;59(Pt 1):8-16.

18. Sangari FJ, Goodman J, Bermudez LE. *Mycobacterium avium* enters intestinal epithelial cells through the apical membrane, but not by the basolateral surface, activates small GTPase Rho and, once within epithelial cells, expresses an invasive phenotype. *Cell Microbiol*. 2000;2(6):561-8.

19. Nightingale SD, Cameron DW, Gordin FM, Sullam PM, Cohn DL, Chaisson RE, et al. Two controlled trials of rifabutin prophylaxis against *Mycobacterium avium* complex infection in AIDS. *N Engl J Med*. 1993;329(12):828-33.

20. Crowle AJ, Dahl R, Ross E, May MH. Evidence that vesicles containing living, virulent *Mycobacterium tuberculosis* or *Mycobacterium avium* in cultured human macrophages are not acidic. *Infect Immun*. 1991;59(5):1823-31.

21. Inderlied CB, Kemper CA, Bermudez LE. The *Mycobacterium avium* complex. *Clin Microbiol Rev*. 1993;6(3):266-310.

22. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity*. 2010;32(5):593-604.

23. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci*. 2008;13:453-61.

24. Hussell T, Bell TJ. Alveolar macrophages: plasticity in a tissue-specific context. *Nat Rev Immunol*. 2014;14(2):81-93.

25. Alber A, Howie SE, Wallace WA, Hirani N. The role of macrophages in healing the wounded lung. *Int J Exp Pathol*. 2012;93(4):243-51.

26. Mitsi E, Kamng'ona R, Rylance J, Solorzano C, Jesus Reine J, Mwandumba HC, et al. Human alveolar macrophages predominantly express combined classical M1 and M2 surface markers in steady state. *Respir Res*. 2018;19(1):66.

27. Benoit M, Desnues B, Mege JL. Macrophage polarization in bacterial infections. *J Immunol*. 2008;181(6):3733-9.

28. Bermudez LE, Young LS, Enkel H. Interaction of *Mycobacterium avium* complex with human macrophages: roles of membrane receptors and serum proteins. *Infect Immun*. 1991;59(5):1697-702.

29. Shamaei M, Mirsaeidi M. Nontuberculous *Mycobacteria*, Macrophages, and Host Innate Immune

Response. *Infect Immun*. 2021;89(8):e0081220.

30. Gomes MS, Florido M, Cordeiro JV, Teixeira CM, Takeuchi O, Akira S, et al. Limited role of the Toll-like receptor-2 in resistance to *Mycobacterium avium*. *Immunology*. 2004;111(2):179-85.
31. Gomes MS, Sousa Fernandes S, Cordeiro JV, Silva Gomes S, Vieira A, Appelberg R. Engagement of Toll-like receptor 2 in mouse macrophages infected with *Mycobacterium avium* induces non-oxidative and TNF-independent anti-mycobacterial activity. *Eur J Immunol*. 2008;38(8):2180-9.
32. Bhatnagar S, Schorey JS. Elevated mitogen-activated protein kinase signalling and increased macrophage activation in cells infected with a glycopeptidolipid-deficient *Mycobacterium avium*. *Cell Microbiol*. 2006;8(1):85-96.
33. Neupane AS, Willson M, Chojnacki AK, Vargas ESCF, Morehouse C, Carestia A, et al. Patrolling Alveolar Macrophages Conceal Bacteria from the Immune System to Maintain Homeostasis. *Cell*. 2020;183(1):110-25 e11.
34. Carvalho NB, Oliveira FS, Duraes FV, de Almeida LA, Florido M, Prata LO, et al. Toll-like receptor 9 is required for full host resistance to *Mycobacterium avium* infection but plays no role in induction of Th1 responses. *Infect Immun*. 2011;79(4):1638-46.
35. Marinho FA, de Paula RR, Mendes AC, de Almeida LA, Gomes MT, Carvalho NB, et al. Toll-like receptor 6 senses *Mycobacterium avium* and is required for efficient control of mycobacterial infection. *Eur J Immunol*. 2013;43(9):2373-85.
36. Lee HJ, Woo Y, Hahn TW, Jung YM, Jung YJ. Formation and Maturation of the Phagosome: A Key Mechanism in Innate Immunity against Intracellular Bacterial Infection. *Microorganisms*. 2020;8(9).
37. Danelishvili L, Bermudez LE. *Mycobacterium avium* MAV_2941 mimics phosphoinositol-3-kinase to interfere with macrophage phagosome maturation. *Microbes Infect*. 2015;17(9):628-37.
38. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, et al. Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science*. 1994;263(5147):678-81.
39. Danelishvili L, Chinison JJJ, Pham T, Gupta R, Bermudez LE. The Voltage-Dependent Anion Channels (VDAC) of *Mycobacterium avium* phagosome are associated with bacterial survival and lipid export in macrophages. *Sci Rep*. 2017;7(1):7007.
40. Houben D, Demangel C, van Ingen J, Perez J, Baldeon L, Abdallah AM, et al. ESX-1-mediated translocation to the cytosol controls virulence of mycobacteria. *Cell Microbiol*. 2012;14(8):1287-98.
41. Sharma V, Verma S, Seranova E, Sarkar S, Kumar D. Selective Autophagy and Xenophagy in Infection and Disease. *Front Cell Dev Biol*. 2018;6:147.
42. Harding CV, Boom WH. Regulation of antigen presentation by *Mycobacterium tuberculosis*: a role for Toll-like receptors. *Nat Rev Microbiol*. 2010;8(4):296-307.
43. Jiao X, Lo-Man R, Guernonprez P, Fiette L, Deriaud E, Burgaud S, et al. Dendritic cells are host cells for mycobacteria in vivo that trigger innate and acquired immunity. *J Immunol*. 2002;168(3):1294-301.
44. Appelberg R, Leal IS, Pais TF, Pedrosa J, Florido M. Differences in resistance of C57BL/6 and C57BL/10 mice to infection by *Mycobacterium avium* are independent of gamma interferon. *Infect Immun*. 2000;68(1):19-23.
45. Daley CL. *Mycobacterium avium* Complex Disease. *Microbiol Spectr*. 2017;5(2).
46. Underhill DM, Ozinsky A, Smith KD, Aderem A. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc Natl Acad Sci U S A*. 1999;96(25):14459-63.
47. Wang T, Lafuse WP, Zwilling BS. Regulation of toll-like receptor 2 expression by macrophages following *Mycobacterium avium* infection. *J Immunol*. 2000;165(11):6308-13.
48. Rodrigues MF, Alves CC, Figueiredo BB, Rezende AB, Wohlfres-Viana S, Silva VL, et al. Tumour necrosis factor receptors and apoptosis of alveolar macrophages during early infection with attenuated and virulent *Mycobacterium bovis*. *Immunology*. 2013;139(4):503-12.
49. Behar SM, Martin CJ, Booty MG, Nishimura T, Zhao X, Gan HX, et al. Apoptosis is an innate defense function of macrophages against *Mycobacterium tuberculosis*. *Mucosal Immunol*. 2011;4(3):279-87.
50. Fratazzi C, Arbeit RD, Carini C, Remold HG. Programmed cell death of *Mycobacterium avium* serovar 4-infected human macrophages prevents the mycobacteria from spreading and induces mycobacterial growth inhibition by freshly added, uninfected macrophages. *J Immunol*. 1997;158(9):4320-7.
51. Keane J, Shurtleff B, Kornfeld H. TNF-dependent BALB/c murine macrophage apoptosis following *Mycobacterium tuberculosis* infection inhibits bacillary growth in an IFN-gamma independent manner. *Tuberculosis (Edinb)*. 2002;82(2-3):55-61.
52. Shundo Y, On R, Matsumoto T, Ouchi H, Fujita M. TNFR1 Mediated Apoptosis Is Protective against *Mycobacterium avium* in Mice. *Microorganisms*. 2023;11(3).
53. Early J, Fischer K, Bermudez LE. *Mycobacterium avium* uses apoptotic macrophages as tools for spreading. *Microb Pathog*. 2011;50(2):132-9.

54. Bermudez LE, Danelishvili L, Babrack L, Pham T. Evidence for genes associated with the ability of *Mycobacterium avium* subsp. *hominissuis* to escape apoptotic macrophages. *Front Cell Infect Microbiol*. 2015;5:63.
55. Lee KI, Whang J, Choi HG, Son YJ, Jeon HS, Back YW, et al. *Mycobacterium avium* MAV2054 protein induces macrophage apoptosis by targeting mitochondria and reduces intracellular bacterial growth. *Sci Rep*. 2016;6:37804.
56. Gomes MS, Florido M, Pais TF, Appelberg R. Improved clearance of *Mycobacterium avium* upon disruption of the inducible nitric oxide synthase gene. *J Immunol*. 1999;162(11):6734-9.
57. Rost LM, Louet C, Bruheim P, Flo TH, Gidon A. Pyruvate Supports RET-Dependent Mitochondrial ROS Production to Control *Mycobacterium avium* Infection in Human Primary Macrophages. *Front Immunol*. 2022;13:891475.
58. Bermudez LE, Young LS. Oxidative and non-oxidative intracellular killing of *Mycobacterium avium* complex. *Microb Pathog*. 1989;7(4):289-98.
59. Bryant JM, Grogono DM, Greaves D, Foweraker J, Roddick I, Inns T, et al. Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. *Lancet*. 2013;381(9877):1551-60.
60. Guidotti TL, Ragain L. Communicating with healthcare providers. *J Water Health*. 2008;6 Suppl 1:53-61.
61. Nishiuchi Y, Maekura R, Kitada S, Tamaru A, Taguri T, Kira Y, et al. The recovery of *Mycobacterium avium*-intracellular complex (MAC) from the residential bathrooms of patients with pulmonary MAC. *Clin Infect Dis*. 2007;45(3):347-51.
62. Prevots DR, Marshall JE, Wagner D, Morimoto K. Global Epidemiology of Nontuberculous Mycobacterial Pulmonary Disease: A Review. *Clin Chest Med*. 2023;44(4):675-721.
63. Tzou CL, Dirac MA, Becker AL, Beck NK, Weigel KM, Meschke JS, et al. Association between *Mycobacterium avium* Complex Pulmonary Disease and Mycobacteria in Home Water and Soil. *Ann Am Thorac Soc*. 2020;17(1):57-62.
64. Maekawa K, Ito Y, Hirai T, Kubo T, Imai S, Tatsumi S, et al. Environmental risk factors for pulmonary *Mycobacterium avium*-intracellular complex disease. *Chest*. 2011;140(3):723-9.
65. Reed C, von Reyn CF, Chamblee S, Ellerbrock TV, Johnson JW, Marsh BJ, et al. Environmental risk factors for infection with *Mycobacterium avium* complex. *Am J Epidemiol*. 2006;164(1):32-40.
66. von Reyn CF, Waddell RD, Eaton T, Arbeit RD, Maslow JN, Barber TW, et al. Isolation of *Mycobacterium avium* complex from water in the United States, Finland, Zaire, and Kenya. *J Clin Microbiol*. 1993;31(12):3227-30.
67. Falkinham JO, 3rd. Nontuberculous mycobacteria in the environment. *Tuberculosis (Edinb)*. 2022;137:102267.
68. O'Brien RJ, Geiter LJ, Snider DE, Jr. The epidemiology of nontuberculous mycobacterial diseases in the United States. Results from a national survey. *Am Rev Respir Dis*. 1987;135(5):1007-14.
69. Olivier KN, Weber DJ, Wallace RJ, Jr., Faiz AR, Lee JH, Zhang Y, et al. Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. *Am J Respir Crit Care Med*. 2003;167(6):828-34.
70. Adjemian J, Olivier KN, Seitz AE, Falkinham JO, 3rd, Holland SM, Prevots DR. Spatial clusters of nontuberculous mycobacterial lung disease in the United States. *Am J Respir Crit Care Med*. 2012;186(6):553-8.
71. Hoffmann C, Leis A, Niederweis M, Plitzko JM, Engelhardt H. Disclosure of the mycobacterial outer membrane: cryo-electron tomography and vitreous sections reveal the lipid bilayer structure. *Proc Natl Acad Sci U S A*. 2008;105(10):3963-7.
72. Brennan PJ, Nikaido H. The envelope of mycobacteria. *Annu Rev Biochem*. 1995;64:29-63.
73. Bendinger B, Rijnaarts HH, Altendorf K, Zehnder AJ. Physicochemical cell surface and adhesive properties of coryneform bacteria related to the presence and chain length of mycolic acids. *Appl Environ Microbiol*. 1993;59(11):3973-7.
74. Steed KA, Falkinham JO, 3rd. Effect of growth in biofilms on chlorine susceptibility of *Mycobacterium avium* and *Mycobacterium intracellulare*. *Appl Environ Microbiol*. 2006;72(6):4007-11.
75. Horsburgh CR, Jr., Gettings J, Alexander LN, Lennox JL. Disseminated *Mycobacterium avium* complex disease among patients infected with human immunodeficiency virus, 1985-2000. *Clin Infect Dis*. 2001;33(11):1938-43.
76. Horsburgh CR, Jr., Selik RM. The epidemiology of disseminated nontuberculous mycobacterial infection in the acquired immunodeficiency syndrome (AIDS). *Am Rev Respir Dis*. 1989;139(1):4-7.
77. Field SK, Fisher D, Cowie RL. *Mycobacterium avium* complex pulmonary disease in patients without HIV infection. *Chest*. 2004;126(2):566-81.
78. de Jong R, Altare F, Haagen IA, Elferink DG, Boer T, van Breda Vriesman PJ, et al. Severe mycobacterial

- and Salmonella infections in interleukin-12 receptor-deficient patients. *Science*. 1998;280(5368):1435-8.
79. Dorman SE, Holland SM. Interferon-gamma and interleukin-12 pathway defects and human disease. *Cytokine Growth Factor Rev*. 2000;11(4):321-33.
 80. Yeh JJ, Wang YC, Sung FC, Kao CH. Rheumatoid arthritis increases the risk of nontuberculosis mycobacterial disease and active pulmonary tuberculosis. *PLoS One*. 2014;9(10):e110922.
 81. Winthrop KL, Chang E, Yamashita S, Iademarco MF, LoBue PA. Nontuberculous mycobacteria infections and anti-tumor necrosis factor-alpha therapy. *Emerg Infect Dis*. 2009;15(10):1556-61.
 82. Winthrop KL, Baxter R, Liu L, Varley CD, Curtis JR, Baddley JW, et al. Mycobacterial diseases and antitumor necrosis factor therapy in USA. *Ann Rheum Dis*. 2013;72(1):37-42.
 83. Brode SK, Jamieson FB, Ng R, Campitelli MA, Kwong JC, Paterson JM, et al. Increased risk of mycobacterial infections associated with anti-rheumatic medications. *Thorax*. 2015;70(7):677-82.
 84. Liu VX, Winthrop KL, Lu Y, Sharifi H, Nasiri HU, Ruoss SJ. Association between Inhaled Corticosteroid Use and Pulmonary Nontuberculous Mycobacterial Infection. *Ann Am Thorac Soc*. 2018;15(10):1169-76.
 85. Dirac MA, Horan KL, Doody DR, Meschke JS, Park DR, Jackson LA, et al. Environment or host?: A case-control study of risk factors for *Mycobacterium avium* complex lung disease. *Am J Respir Crit Care Med*. 2012;186(7):684-91.
 86. Loebinger MR, Quint JK, van der Laan R, Obradovic M, Chawla R, Kishore A, et al. Risk Factors for Nontuberculous Mycobacterial Pulmonary Disease: A Systematic Literature Review and Meta-Analysis. *Chest*. 2023;164(5):1115-24.
 87. Chen YM, Lin CH, Chen HH, Chao WC, Chen DY, Lin CC, et al. Risk of mycobacterial disease among cancer patients: A population-based cohort study in a TB endemic area. *Cancer Epidemiol*. 2019;59:64-70.
 88. Chan ED, Iseman MD. Slender, older women appear to be more susceptible to nontuberculous mycobacterial lung disease. *Gend Med*. 2010;7(1):5-18.
 89. Martiniano SL, Nick JA. Nontuberculous mycobacterial infections in cystic fibrosis. *Clin Chest Med*. 2015;36(1):101-15.
 90. Matesanz Lopez C, Loras Gallego C, Cacho Calvo J, Thuissard Vasallo IJ, Rio Ramirez MT. Patients with non-tuberculous mycobacteria in respiratory samples: a 5-year epidemiological study. *Rev Esp Quimioter*. 2021;34(2):120-5.
 91. Munjal S, Munjal S, Gao J, Venketaraman V. Exploring Potential COPD Immunosuppression Pathways Causing Increased Susceptibility for MAC Infections among COPD Patients. *Clin Pract*. 2021;11(3):619-30.
 92. Sterling TR, Moore RD, Graham NM, Astemborski J, Vlahov D, Chaisson RE. *Mycobacterium tuberculosis* infection and disease are not associated with protection against subsequent disseminated *M. avium* complex disease. *AIDS*. 1998;12(12):1451-7.
 93. Kim SJ, Yoon SH, Choi SM, Lee J, Lee CH, Han SK, et al. Characteristics associated with progression in patients with nontuberculous mycobacterial lung disease : a prospective cohort study. *Bmc Pulm Med*. 2017;17(1):5.
 94. Mustafa T. Does leptin have a role in immunity to tuberculosis? *Indian J Med Res*. 2008;128(6):691-3.
 95. Naylor C, Petri WA, Jr. Leptin Regulation of Immune Responses. *Trends Mol Med*. 2016;22(2):88-98.
 96. Tasaka S, Hasegawa N, Nishimura T, Yamasawa W, Kamata H, Shinoda H, et al. Elevated serum adiponectin level in patients with *Mycobacterium avium*-intracellulare complex pulmonary disease. *Respiration*. 2010;79(5):383-7.
 97. Luo Y, Liu M. Adiponectin: a versatile player of innate immunity. *J Mol Cell Biol*. 2016;8(2):120-8.
 98. Park HE, Lee W, Choi S, Jung M, Shin MK, Shin SJ. Modulating macrophage function to reinforce host innate resistance against *Mycobacterium avium* complex infection. *Front Immunol*. 2022;13:931876.
 99. Lake MA, Ambrose LR, Lipman MC, Lowe DM. "Why me, why now?" Using clinical immunology and epidemiology to explain who gets nontuberculous mycobacterial infection. *BMC Med*. 2016;14:54.
 100. Mirsaeidi M, Hadid W, Ericsson B, Rodgers D, Sadikot RT. Non-tuberculous mycobacterial disease is common in patients with non-cystic fibrosis bronchiectasis. *Int J Infect Dis*. 2013;17(11):e1000-4.
 101. Kim RD, Greenberg DE, Ehrmantraut ME, Guide SV, Ding L, Shea Y, et al. Pulmonary nontuberculous mycobacterial disease: prospective study of a distinct preexisting syndrome. *Am J Respir Crit Care Med*. 2008;178(10):1066-74.
 102. Prevots DR, Shaw PA, Strickland D, Jackson LA, Raebel MA, Bosky MA, et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. *Am J Respir Crit Care Med*. 2010;182(7):970-6.
 103. Tsuyuguchi K, Suzuki K, Matsumoto H, Tanaka E, Amitani R, Kuze F. Effect of oestrogen on *Mycobacterium avium* complex pulmonary infection in mice. *Clin Exp Immunol*. 2001;123(3):428-34.

104. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007;175(4):367-416.
105. McShane PJ, Glassroth J. Pulmonary Disease Due to Nontuberculous Mycobacteria: Current State and New Insights. *Chest*. 2015;148(6):1517-27.
106. Tortoli E. Clinical manifestations of nontuberculous mycobacteria infections. *Clin Microbiol Infect*. 2009;15(10):906-10.
107. Johnson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections. *J Thorac Dis*. 2014;6(3):210-20.
108. Pulmonary disease caused by *Mycobacterium avium*-intracellulare in HIV-negative patients: five-year follow-up of patients receiving standardised treatment. *Int J Tuberc Lung Dis*. 2002;6(7):628-34.
109. Prince DS, Peterson DD, Steiner RM, Gottlieb JE, Scott R, Israel HL, et al. Infection with *Mycobacterium avium* complex in patients without predisposing conditions. *N Engl J Med*. 1989;321(13):863-8.
110. Kaplan JE, Masur H, Holmes KK, Usphs, Infectious Disease Society of A. Guidelines for preventing opportunistic infections among HIV-infected persons--2002. Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. *MMWR Recomm Rep*. 2002;51(RR-8):1-52.
111. Ford MB, Okulicz JF, Salinas JR, Kiley JL. Epidemiology, clinical characteristics, and outcomes of nontuberculous mycobacterial skin, soft tissue, and bone infections from a single center over a 10-year period. *J Clin Tuberc Other Mycobact Dis*. 2023;33:100403.
112. Hermansen TS, Thomsen VO, Lillebaek T, Ravn P. Non-tuberculous mycobacteria and the performance of interferon gamma release assays in Denmark. *PLoS One*. 2014;9(4):e93986.
113. McNabb A, Eisler D, Adie K, Amos M, Rodrigues M, Stephens G, et al. Assessment of partial sequencing of the 65-kilodalton heat shock protein gene (*hsp65*) for routine identification of *Mycobacterium* species isolated from clinical sources. *J Clin Microbiol*. 2004;42(7):3000-11.
114. Xu K, Bi S, Ji Z, Hu H, Hu F, Zheng B, et al. Distinguishing nontuberculous mycobacteria from multidrug-resistant *Mycobacterium tuberculosis*, China. *Emerg Infect Dis*. 2014;20(6):1060-2.
115. Grigg C, Jackson KA, Barter D, Czaja CA, Johnston H, Lynfield R, et al. Epidemiology of Pulmonary and Extrapulmonary Nontuberculous Mycobacteria Infections at 4 US Emerging Infections Program Sites: A 6-Month Pilot. *Clin Infect Dis*. 2023;77(4):629-37.
116. Mercaldo RA, Marshall JE, Cangelosi GA, Donohue M, Falkinham JO, 3rd, Fierer N, et al. Environmental risk of nontuberculous mycobacterial infection: Strategies for advancing methodology. *Tuberculosis (Edinb)*. 2023;139:102305.
117. Thomson R, Donnan E, Konstantinos A. Notification of Nontuberculous Mycobacteria: An Australian Perspective. *Ann Am Thorac Soc*. 2017;14(3):318-23.
118. Dahl VN, Molhave M, Floe A, van Ingen J, Schon T, Lillebaek T, et al. Global trends of pulmonary infections with nontuberculous mycobacteria: a systematic review. *Int J Infect Dis*. 2022;125:120-31.
119. Hoefsloot W, van Ingen J, Andrejak C, Angeby K, Bauriaud R, Bemer P, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. *Eur Respir J*. 2013;42(6):1604-13.
120. van Ingen J, Hoefsloot W, Dekhuijzen PN, Boeree MJ, van Soolingen D. The changing pattern of clinical *Mycobacterium avium* isolation in the Netherlands. *Int J Tuberc Lung Dis*. 2010;14(9):1176-80.
121. Al-Houqani M, Jamieson F, Mehta M, Chedore P, May K, Marras TK. Aging, COPD, and other risk factors do not explain the increased prevalence of pulmonary *Mycobacterium avium* complex in Ontario. *Chest*. 2012;141(1):190-7.
122. Brode SK, Daley CL, Marras TK. The epidemiologic relationship between tuberculosis and non-tuberculous mycobacterial disease: a systematic review. *Int J Tuberc Lung Dis*. 2014;18(11):1370-7.
123. Wallace RJ, Jr., Brown BA, Griffith DE, Girard WM, Murphy DT. Clarithromycin regimens for pulmonary *Mycobacterium avium* complex. The first 50 patients. *Am J Respir Crit Care Med*. 1996;153(6 Pt 1):1766-72.
124. van Ingen J, Aksamit T, Andrejak C, Bottger EC, Cambau E, Daley CL, et al. Treatment outcome definitions in nontuberculous mycobacterial pulmonary disease: an NTM-NET consensus statement. *Eur Respir J*. 2018;51(3).
125. Kwon YS, Koh WJ, Daley CL. Treatment of *Mycobacterium avium* Complex Pulmonary Disease. *Tuberc Respir Dis (Seoul)*. 2019;82(1):15-26.
126. Heifets LB, Iseman MD. Individualized therapy versus standard regimens in the treatment of *Mycobacterium avium* infections. *Am Rev Respir Dis*. 1991;144(1):1-2.
127. Dutt AK, Stead WW. Long-term results of medical treatment in *Mycobacterium intracellulare* infection. *Am J Med*. 1979;67(3):449-53.

128. Yeager H, Jr., Raleigh JW. Pulmonary disease due to *Mycobacterium intracellulare*. *Am Rev Respir Dis*. 1973;108(3):547-52.
129. Adjemian J, Prevots DR, Gallagher J, Heap K, Gupta R, Griffith D. Lack of adherence to evidence-based treatment guidelines for nontuberculous mycobacterial lung disease. *Ann Am Thorac Soc*. 2014;11(1):9-16.
130. Tanaka E, Kimoto T, Tsuyuguchi K, Watanabe I, Matsumoto H, Niimi A, et al. Effect of clarithromycin regimen for *Mycobacterium avium* complex pulmonary disease. *Am J Respir Crit Care Med*. 1999;160(3):866-72.
131. Morimoto K, Namkoong H, Hasegawa N, Nakagawa T, Morino E, Shiraishi Y, et al. Macrolide-Resistant *Mycobacterium avium* Complex Lung Disease: Analysis of 102 Consecutive Cases. *Ann Am Thorac Soc*. 2016;13(11):1904-11.
132. Griffith DE, Brown-Elliott BA, Langsjoen B, Zhang Y, Pan X, Girard W, et al. Clinical and molecular analysis of macrolide resistance in *Mycobacterium avium* complex lung disease. *Am J Respir Crit Care Med*. 2006;174(8):928-34.
133. Nguyen MH, Daley CL. Treatment of *Mycobacterium avium* Complex Pulmonary Disease: When Should I Treat and What Therapy Should I Start? *Clin Chest Med*. 2023;44(4):771-83.
134. Dartois V, Dick T. Therapeutic developments for tuberculosis and nontuberculous mycobacterial lung disease. *Nat Rev Drug Discov*. 2024;23(5):381-403.
135. Siddiqi SH, Heifets LB, Cynamon MH, Hooper NM, Laszlo A, Libonati JP, et al. Rapid broth microdilution method for determination of MICs for *Mycobacterium avium* isolates. *J Clin Microbiol*. 1993;31(9):2332-8.
136. Woods GL, Brown-Elliott BA, Conville PS, Desmond EP, Hall GS, Lin G, et al. Susceptibility Testing of *Mycobacteria*, *Nocardiae*, and Other Aerobic Actinomycetes. CLSI Standards: Guidelines for Health Care Excellence. 2nd ed. Wayne (PA)2011.
137. Brown-Elliott BA, Nash KA, Wallace RJ, Jr. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. *Clin Microbiol Rev*. 2012;25(3):545-82.
138. Cowman S, Burns K, Benson S, Wilson R, Loebinger MR. The antimicrobial susceptibility of nontuberculous mycobacteria. *J Infect*. 2016;72(3):324-31.
139. Research Committee of the British Thoracic S. First randomised trial of treatments for pulmonary disease caused by *M avium intracellulare*, *M malmoense*, and *M xenopi* in HIV negative patients: rifampicin, ethambutol and isoniazid versus rifampicin and ethambutol. *Thorax*. 2001;56(3):167-72.
140. van Ingen J, Boeree MJ, van Soolingen D, Mouton JW. Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. *Drug Resist Updat*. 2012;15(3):149-61.
141. Rose RM, Fuglestad JM, Remington L. Growth inhibition of *Mycobacterium avium* complex in human alveolar macrophages by the combination of recombinant macrophage colony-stimulating factor and interferon-gamma. *Am J Respir Cell Mol Biol*. 1991;4(3):248-54.

