



# Nontuberculous mycobacterial lung disease: ecology, microbiology, pathogenesis, and antibiotic resistance mechanisms

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## ABSTRACT

The incidence and prevalence of lung disease caused by nontuberculous mycobacteria (NTM) are increasing worldwide. Environmental sources of NTM include water, soil, and dust, and the acquisition of NTM infection depends on sufficient environmental exposure, host susceptibility factors such as immunocompromised status or cystic fibrosis, and mycobacterial virulence factors. The development of molecular methods has allowed the characterization of new species and the identification of NTM to the precise species and subspecies levels. *Mycobacterium avium* complex, *M. abscessus* complex, and *M. kansasii* are the most frequently identified organisms causing lung disease. Susceptibility to disease is incompletely understood, and thus, it is unclear what preventative measures may be effective. Additionally, NTM have natural and acquired resistance mechanisms to several antibiotics. Better understanding of the ecology, pathogenesis, and mycobacterial genetics and antibiotic resistance mechanisms is essential for preventing NTM infections and developing new regimens for effective treatment.

**Keywords:** Drug resistance, microbial; Ecology; Epidemiology; Etiology; Nontuberculous mycobacteria

## INTRODUCTION

The term “nontuberculous mycobacteria” (NTM) generally refers to mycobacteria other than the *Mycobacterium tuberculosis* complex and *M. leprae* [1]. NTM are ubiquitous in the environment, including household water, natural water sources, and soil [2]. Human disease due to NTM is classified into four distinct clinical syndromes: chronic pulmonary disease, lymphadenitis, cutaneous disease, and disseminated disease. Of these, chronic pulmonary disease is the syndrome most commonly encountered clinically [1]. The incidence and prevalence of NTM lung disease are increasing worldwide. Common causative organisms of pulmonary infection are the slowly growing mycobacteria (SGM), including *M. avium* complex (MAC) and *M. kansasii*, and the rapidly growing mycobacteria (RGM) including *M. abscessus* complex (MABC).

Clinical aspects of NTM lung disease, such as epidemiology, diagnosis, and treatment, were recently reviewed [3-5]. In this article, therefore, we review the ecology, microbiology, patho-

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genesis, and antibiotic resistance mechanisms of NTM lung disease.

## ECOLOGY AND INFECTION ROUTE

Understanding the physiology and structural features of NTM is the key to understanding their ecology, routes of transmission, and resistance to antibiotics and disinfectants. The lipid-rich outer envelope results in NTM cells that are impermeable to hydrophilic nutrients and resistant to heavy metals, disinfectants, and antibiotics [6-8]. Furthermore, the extreme hydrophobicity of the NTM envelope results in cells that prefer surface attachment during growth (i.e., biofilm formation) in the environment and in tissue rather than growing in a planktonic state. Growth in biofilms further increases the resistance of NTM cells to disinfectants and antibiotics [9]. The combination of the impermeable envelope and the low number (1 or 2) of ribosomal RNA (rRNA) operons contributes to the slow growth of NTM (one generation per day) [10].

Unlike members of the *M. tuberculosis* complex, the NTM are free-living bacteria and not obligate parasites of humans. NTM are normal inhabitants of natural soils and water and thereby enter drinking-water treatment plants. From surface waters, the particulate-attached NTM enter the water treatment plants. NTM are approximately 10 to 100 times more resistant to chlorine, chloramine, chlorine dioxide, and ozone compared to *Escherichia coli* [11], and as a result, NTM can survive in water distribution systems [12]. In these distribution systems, the hydrophobic NTM attach to pipe surfaces and form biofilms [13], which prevent these slowly growing bacteria from being washed out of the systems. The major determinants of NTM growth and persistence in water distribution systems are the levels of disinfectant and of reduced organic carbon, but NTM are disinfectant-resistant compared to many other bacteria, and in the absence of competitors, the NTM can utilize all the available organic carbon [12]. From the distribution systems, NTM enter the plumbing of homes, hospitals, long-term care facilities, and office buildings. NTM numbers increase appreciably in water heating systems, and thereby, higher numbers enter premise plumbing. Premise plumbing is an ideal habitat for NTM: the water is warm, there are plenty of surfaces for biofilm formation, and there are few competitors for the limited nutrients. Although NTM can be recovered from premise plumbing water samples in the range of 100 to 1,000 colony-forming unit (CFU)/mL, many more NTM cells are present in biofilms (10,000 CFU/cm<sup>2</sup>) [12]. The ability of NTM to grow at low oxy-

gen concentrations means that they can persist and grow in low oxygen habitats such as biofilms. Also, NTM are relatively resistant to high temperature [14]. The time necessary to kill 90% of *M. avium* cells is 1,000 minutes at 50°C, 54 minutes at 55°C, and 4 minutes at 60°C [14]. For *M. intracellulare*, the time necessary to kill 90% of cells is 550 minutes at 50°C, 24 minutes at 55°C, and 1.5 minutes at 60°C [14]. NTM are frequently isolated from household plumbing where the water heater temperature is 50°C or lower [15].

The major route of NTM infection leading to pulmonary disease is via aerosols. *M. avium* and *M. intracellulare* are readily aerosolized from water, and their cell concentrations in aerosolized droplets can be some 1,000- to 10,000-fold higher than in a cell suspension [16]. Aerosolized NTM cells are further associated with droplets small enough ( $\leq 1 \mu\text{m}$  in diameter) to enter the alveoli [16]. Although individuals are infected by NTM in aerosols generated by splashing water in showers or sinks, it is the NTM in biofilms that ensure the persistence of NTM in premise plumbing. Furthermore, NTM in biofilms, as is the case with other bacteria, are even more resistant to disinfectants than those in water [17]. The high prevalence of NTM in premise plumbing was confirmed in a study of showerheads across the United States, which found that 70% of showerheads had NTM [18]. *M. avium* cells in a showerhead were shown to be identical by DNA fingerprinting to *M. avium* cells isolated from the patient using the shower [13]. In another study, half of 30 households of NTM-infected patients had NTM species that were identical by DNA fingerprinting to the species infecting them [15]. Wallace et al. [19] evaluated MAC household water isolates from three published studies and 37 additional MAC respiratory disease patients. The water and biofilm samples were obtained from 752 individual sites in 80 households of 73 NTM patients. Although *M. intracellulare* was identified by nonsequencing methods in 41 household water/biofilm samples, internal transcribed spacer sequencing revealed that none of the samples contained *M. intracellulare* and that 30 samples were *M. chimaera*, eight were other MAC X species, and three were *M. avium* [19]. In comparison, *M. avium* was recovered from 144 (19.1%, 144/752) water/biofilm samples [19]. These results indicate that, although household water is a probable source of infection by *M. chimaera* and some other MAC species, *M. intracellulare* lung disease in the United States is acquired from environmental sources other than household water.

Although not as emphasized as waterborne NTM infection, NTM in soil have also been linked to pulmonary disease. Dusts generated during the handling of potting soil are rich

in NTM [20], and a substantial proportion of dustborne NTM are associated with particles small enough ( $\leq 1$  to  $2 \mu\text{m}$  in diameter) to enter lung alveoli [20]. In a comparison of NTM isolates from patients with those from dusts generated by soil samples that the patients used in gardening, five of 11 patients were infected with genetically the same isolate that was found in the soils [20]. In Japan, NTM were detected in soil and house-dust samples [21]. MAC strains were recovered from 48.9% of residential soil samples in households of 100 pulmonary MAC patients and 35 non-infected control subjects. The frequency of MAC recovery did not differ among soil types [21]. In addition, a subsequent report showed that high exposure to soil ( $\geq 2$  hr/wk) was associated with polyclonal and mixed mycobacterial MAC infections in pulmonary MAC disease patients [22].

In general, though, the modes of NTM transmission to humans have not been well defined. Unlike tuberculosis, person-to-person transmission has not been convincingly demonstrated. However, studies on potential transmission of NTM between cystic fibrosis patients have been aided in recent years by the implementation of whole-genome sequencing (WGS), which can identify strains with an extremely high level of accuracy. The first application of WGS for an NTM survey was performed by Bryant et al. [23] who investigated how *M. abscessus* subsp. *massiliense* was acquired by individuals with cystic fibrosis. *M. abscessus* subsp. *massiliense* isolates from 31 patients with cystic fibrosis in the United Kingdom between 2007 and 2011 were subjected to WGS and phylogenetic analysis. Isolates from 11 patients with *M. abscessus* subsp. *massiliense* were nearly identical, differing by fewer than ten base pairs, which strongly indicated between-patient transmission. Moreover, additional analysis provided evidence for transmission of drug-resistant strains. However, the study was inconclusive as to whether transmission occurred directly between patients or indirectly, such as via contamination of patient treatment facilities [23]. In another study, 27 *M. abscessus* isolates from 20 pediatric cystic fibrosis patients were examined by WGS, but most isolates were unrelated, differing by at least 34 single nucleotide polymorphisms (SNPs) from any other isolate [24]. The strongest evidence for cross-transmission was found for two siblings whose isolates differed by a maximum of 17 SNPs [24]. However, in another study by Bryant and colleagues [25], WGS was used to examine a global collection of clinical isolates, including 1,080 clinical isolates of *M. abscessus* obtained from 517 patients in United Kingdom cystic fibrosis clinics and regional reference laboratories in other countries,

e.g., cystic fibrosis centers in the United States, the Republic of Ireland, Europe, and Australia. This study showed that the majority of *M. abscessus* infections are acquired through transmission, potentially via fomites and aerosols, of recently emerged, dominant circulating clones that have spread globally in cystic fibrosis patients [25].

To explore the mechanisms associated with the global spread of infection and NTM transmission routes, an epidemiological, population-wide genotyping survey would be useful. WGS will improve NTM surveys, allowing the mechanisms for the global spread of NTM disease to become clearer in the near future.

## MICROBIOLOGY

### Runyon classification

Historically, NTM have been divided by Runyon criteria according to their differences in growth rate on solid culture and in pigment formation [26-28]. On the basis of these criteria, NTM have been divided into four groups (Table 1). Runyon I, II, and III organisms consist of SGM (defined as having colonies maturing in more than 1 week), whereas Runyon IV consists of RGM (defined as having colonies maturing in less than 1 week).

Runyon I organisms, called “photochromogens,” produce a yellow or orange pigment when exposed to light and include *M. kansasii*, *M. marinum*, and *M. simiae* species. Runyon II organisms, called “scotochromogens,” produce a yellow-orange pigment regardless of whether they are exposed to dark or light, and this group includes *M. scrofulaceum*, *M. szulgai*, and *M. gordonae* species. Runyon III organisms are “nonchromogens” and do not produce pigment under any culture condition; the group includes MAC, *M. ulcerans*, *M. xenopi*, *M. malmoense*, *M. terrae* complex, *M. haemophilum*, and *M. genavense* species. Runyon IV organisms, in common with Runyon III organisms, do not produce pigment. However, Runyon IV species have faster growth rates. This group includes MABC, *M. chelonae*, and *M. fortuitum* complex (Table 1) [29,30].

Among the more than 170 known NTM species, *M. kansasii* (Runyon I), MAC (Runyon III), and MABC (Runyon IV) were reported as major pathogens in NTM lung disease and ones that continue to emerge worldwide [31-33].

### *M. avium* complex

MAC are widely distributed and the most common cause of NTM lung disease worldwide, including in Korea [31,32,34-

**Table 1.** Classification of slowly growing mycobacteria and rapidly growing mycobacteria of NTM according to Runyon criteria

Type of growth	Runyon classifications	Species
Slowly growing mycobacteria (colonies visible after more than 7 days)	Runyon I (photochromogens)	<i>Mycobacterium kansasii</i> <sup>a)</sup> <i>M. marinum</i> <i>M. simiae</i>
	Runyon II (scotochromogens)	<i>M. scrofulaceum</i> <i>M. szulgai</i> <i>M. gordonae</i>
	Runyon III (nonchromogens)	<i>M. avium</i> complex <sup>a)</sup> <i>M. genavense</i> <i>M. haemophilum</i> <i>M. malmoense</i> <i>M. terrae</i> complex <i>M. ulcerans</i> <i>M. xenopi</i>
Rapidly growing mycobacteria (colonies visible in fewer than 7 days)	Runyon IV	<i>M. abscessus</i> complex <sup>a)</sup> <i>M. chelonae</i> <i>M. fortuitum</i> complex

NTM, nontuberculous mycobacteria.

<sup>a)</sup>Major NTM pathogen causing human lung disease worldwide.

37]. In the early 1980s, the complex was originally composed of two primary species, *M. avium* and *M. intracellulare*, which cannot be differentiated through traditional physical and biochemical methods [38]. However, with the development of molecular identification methods, several new species that are closely related to *M. intracellulare* have been identified: *M. chimaera*, *M. colombiense*, *M. arosiense*, *M. vulneris*, *M. marseillense*, *M. timonense*, *M. bouchedurhonense*, *M. mantenii*, *M. yongonense*, *M. paraintracellulare*, and “*M. indicus pranii*” [39-49]. In addition, *M. avium* has been further subdivided into four subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *silvaticum* [50,51].

MAC are commonly and widely isolated from soil, house dust, and water [16,52-56]. Many studies also report detection of MAC in sputum, sinus, and variable water samples [15,57-61]. Interestingly, previous studies have suggested household water (especially from bathroom showers) as a source of MAC in lung disease [13,15,18,57,58]. However, a recent epidemiological investigation suggested that *M. intracellulare* lung disease is acquired from environmental sources other than household water [19], indicating that different MAC subspecies may have different habitats.

### *M. kansasii*

*M. kansasii* is the second most common cause of NTM lung disease after MAC in some countries [1,33,62]. However, in

Korea, *M. kansasii* is a relatively uncommon cause of NTM lung disease [4,63,64]. In addition, infection by *M. kansasii* may occur via an aerosol route, with low infectivity in regions of endemicity [65]. Unlike other common NTM, *M. kansasii* is infrequently isolated from natural water sources or soil [66]. Although it is not known with certainty, the major reservoir of *M. kansasii* appears to be tap water [67].

Through various molecular analyses, *M. kansasii* has been divided into five subtypes [68,69]. Among *M. kansasii* clinical isolates, subtype I is the predominant subtype and is responsible for most human infections in the United States, Europe, and Japan. However, studies on the distribution and prevalence of subtypes of *M. kansasii* in Korea are very insufficient.

### *M. abscessus* complex

Among RGM, MABC are the most common mycobacteria involved in pulmonary infections and have emerged as important human pathogens responsible for a wide spectrum of soft-tissue infections [70-72]. Separated from the *M. chelonae* group in 1992, MABC was recently differentiated into three subspecies (*M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii*) [73].

MABC is also extensively found in soil and water, in common with other NTM [74]. Previous studies revealed that MABC infections can be acquired in the community or in the hospital environment [75,76]. In the community environment, water supply systems are regarded as likely sources of

MABC infection, and in the hospital environment, contamination of medical devices and water are considered to be potential sources of MABC infections [77]. Although MAC are the most common NTM species responsible for disease, the natural resistance of MABC to many antibiotics makes infections with the latter more difficult to treat [78-81].

### Genetic difference between SGM and RGM

*M. tuberculosis* and *M. leprae*, which cause the serious human diseases of tuberculosis and leprosy, respectively, are SGM [82]. MAC and *M. kansasii* are also SGM and are major pulmonary pathogens worldwide [1]. It is believed that SGM evolved from RGM [83,84].

Since the 2000s, there has been an increasing number of studies analyzing the genetic differences between SGM and RGM using genome sequencing. For example, ESX-5 (VMware Inc., Palo Alto, CA, USA), one of the type VII secretion systems (T7S) that secrete proteins across the unusual mycobacterial cell envelope, is only present in SGM and is a marker for distinguishing between SGM and RGM [83,85,86].

More recently, Wee and colleagues [87] compared the genomes of 28 mycobacterial species to improve understanding of the distinction between RGM and SGM. The SGM were found to have lost genes (*LivFGMH* operon, *shaACDEFG* genes, and the *MspA* porin gene) associated with growth rates in other bacteria. Furthermore, among RGM, *M. abscessus* had a high number of species-specific genes, some associated with quorum sensing and which might be related to bacterial survival. Based on these analyses, the authors suggested that the mycobacterial genus has undergone a series of gene gain and loss events in the course of its evolution, which likely enabled the various species to adapt to different environments and led to the evolution of the present-day SGM and RGM groups [87].

## PATHOGENESIS

### Host factors

Despite the fact that NTM are widespread in the environment and that exposure to these organisms is inevitable, NTM lung disease is relatively uncommon, suggesting that normal host defense mechanisms are sufficient to prevent NTM infection and that patients who develop NTM lung disease likely have specific susceptibility factors that make them vulnerable to these infections [88].

A number of diseases associated with structural lung damage have been recognized as predisposing patients to NTM

lung disease. Chronic obstructive pulmonary disease (COPD) has frequently been associated with NTM lung disease [89-92]. COPD is a chronic, progressive lung disease characterized by airflow limitation with poor reversibility. Lung injury, triggered most often by smoking, causes inflammation, tissue destruction, and remodeling of elastin and collagen, with occlusion of small airways by narrowing, obliteration, and mucus plugs [93]. The heightened susceptibility to infection was demonstrated in a prospective cohort study of COPD patients with exacerbations where 22% of subjects were culture-positive for NTM [94]. The extent to which NTM infection may promote COPD has been investigated and might partly explain the apparent high frequency of mycobacterial isolation from COPD patients [95].

Cystic fibrosis, an autosomal recessive disease due to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), is strongly associated with bronchiectasis and pulmonary NTM infections, with prevalence rates of NTM in cystic fibrosis patients ranging from 6.6% to 13.7% [96-99]. However, when pulmonary NTM infection was investigated in patients with cystic fibrosis who were more than 40 years of age, the rates were closer to 50% [97].

In recent years, the relationship between NTM and noncystic fibrosis bronchiectasis has been further elucidated [100]. NTM can cause bronchiectasis by destroying the bronchial anatomy, and bronchiectasis can predispose patients to NTM colonization/disease due to impaired host local defenses [101,102]. NTM lung disease in bronchiectasis can also be complicated by coinfection with other bacteria, especially *Pseudomonas aeruginosa* [103], and fungi, including *Aspergillus* species [104]. Bronchiectasis and other forms of lung damage caused by concurrent or prior pulmonary tuberculosis are also associated with NTM infection; whether this predisposition is due to local impairment in host defenses from lung damage or to a common underlying immunological defect is unknown [105,106]. NTM are frequently found in respiratory specimens from patients with pulmonary tuberculosis, but the clinical significance of the NTM in this setting is unclear [107-109]. In a systematic review, the overall prevalence of NTM among patients with bronchiectasis was 9.3%. Subgroup analysis revealed that the prevalence was higher in studies with a larger sample size, year of study of 2002 or afterwards, and Asian locations [110].

NTM lung disease are common in patients with primary ciliary dyskinesia, an increasingly recognized recessive disorder affecting ciliary function of the respiratory tract, sperm tails, cilia of the embryonic node, and the fallopian tubes [111].



Clinical manifestations of primary ciliary dyskinesia include recurrent respiratory tract infections, chronic sinusitis, rhinitis, otitis media, infertility, and laterality defects such as situs inversus totalis or heterotaxy [112]. More than 15% of patients with primary ciliary dyskinesia have at least one sputum culture positive for NTM infection [111].

However, it is increasingly recognized that NTM lung disease may occur in patients without underlying lung disease. This discovery was first described in the early 1990s in a cohort of postmenopausal women who had no predisposing lung disease [113]. Lady Windermere syndrome was the eponym applied to the condition of these patients, who tend to be taller and leaner with more scoliosis, pectus excavatum, and mitral valve prolapse than their peers [114,115]. These patients have increased prevalence of heterozygous mutations in CFTR (up to 50% of cases), usually without a clinical diagnosis of cystic fibrosis [114,116]. Several obvious family clusters in a dominant pattern indicate that genetic factors probably underlie at least some cases of NTM lung disease [117]. Defects that overlap with primary ciliary dyskinesia including impaired nasal nitric oxide concentration, low ciliary-beat frequency, and impaired Toll-like responses in respiratory epithelial cells were also reported in these patients [118]. Despite extensive study, no reproducible defect in immune function in adult patients with isolated lung disease has been found [114,115,119]. The absence of consistent immunological abnormalities, the late age of disease onset, and the lack of disseminated disease in patients with extensive and fatal pulmonary disease (and *vice versa*) suggest that NTM lung disease in these patients is not due to a major underlying immune defect. Recent whole-exome studies suggest that patients with Lady Windermere syndrome have a complex condition involving additive genetic variants in immune, ciliary, connective tissue, and CFTR genes [120].

### Host genetic susceptibility

Host defense against mycobacteria depends on the action of cell-mediated immunity, effected by interactions between the myeloid (monocytes, macrophages, and dendritic cells) and the lymphoid cells (T cells and natural killer cells) [121]. The critical effector cell for controlling NTM is the macrophage, which ingests the mycobacteria. Once engulfed by the macrophage, the bacteria's fate, whether destruction, persistence, or replication, is determined by the cell's state of immune activation, with this activation determined by interactions between cells in the TH1 pathway and their associated cytokines, particularly the interleukin 12 (IL-12)/interferon

$\gamma$  (IFN- $\gamma$ ) axis. Mononuclear phagocytes engulf mycobacteria and produce IL-12, which stimulates T cells and natural killer cells through the IL-12 receptor [121]. STAT4 (signal transducer and activator of transcription 4) is activated, leading to induction of IFN- $\gamma$  production, which binds to its receptor, causing activation and differentiation of macrophages with further upregulation of the expression of IL-12 and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) [122,123]. The activated macrophages are then able to kill intracellular organisms such as NTM. Numerous other cytokines (e.g., IL-18, IL-23, and IL-29), receptors (e.g., vitamin D receptor), and unidentified cofactors may also be important.

TH1 immunity can be triggered through Toll-like receptors (TLRs), and mice lacking TLR2 were more susceptible to *M. avium* infection [124,125]. TLR9 signaling may also play a small role. Recent evidence suggests that NTM initially trigger immunity via the AIM2 (absent in melanoma 2)-inflammasome, whereas *M. tuberculosis* does so via NLRP3 (nucleotide-binding domain, leucine rich family [NLR], pyrin containing 3 gene) [126].

In addition to CD4 cells, IL-12 was also implicated in TNF- $\alpha$  production by NK cells in *M. avium* infections [127]. An early role for IL-6 was suggested, as was a late response involving IL-10 [128]. More recently, a role for TNF- $\alpha$  has been illustrated by infections occurring in patients treated with anti-TNF- $\alpha$  biologic reagents for rheumatoid arthritis [129,130].

Mendelian susceptibility to mycobacterial disease comprises several disorders related to IFN- $\gamma$  production deficits or poor responsiveness to IFN- $\gamma$  due to receptor abnormalities or deficiencies [131,132]. There are at least seven autosomal mutations and two X-linked mutations that have been associated with disseminated disease, usually occurring in childhood [121]. Three of the autosomal genes are implicated in the control of IFN- $\gamma$  production: IL12B, IL12RB1, and ISG15 (interferon-stimulated gene 15) [121,133]. Four other autosomal genes are implicated in IFN- $\gamma$  responsiveness: IFNGR1 (interferon  $\gamma$  receptor 1), IFNGR2, STAT1, and IRF8 (interferon regulatory factor 8) [134]. Specific mutations in IKBKG (inhibitor of nuclear factor  $\kappa$ B kinase subunit  $\gamma$ ) and CYBB (cytochrome b  $\alpha$  [CYBA] and  $\beta$ ) cause X-linked Mendelian susceptibility to mycobacterial disease. Approximately 80% of genetically diagnosed cases are caused by IFN- $\gamma$  receptor or IL-12 receptor deficiency; however, approximately half of all patients with disseminated NTM have no discernible defects in the IL-12/IFN- $\gamma$  axis [121]. GATA2 (GATA binding protein 2) is a transcription factor implicated in early hemopoietic, lymphatic, and vascular development, and deficiency gives rise

to a wide range of disease phenotypes previously called the “monocytopenia with *M. avium* complex syndrome” [121]. Infections can present from age 3 to 80 years with profoundly decreased or absent circulating monocytes, dendritic cells, natural killer cells, and B cells [121]. Anti-IFN- $\gamma$  autoantibodies cause an acquired susceptibility to NTM [135,136], and all cases present in adults, usually with high-titer neutralizing antibodies against IFN- $\gamma$ . Most patients outside of Asia are female, but the gender distribution in Asia is equal. These patients often present with other intracellular pathogens but, interestingly, not *M. tuberculosis* [135,136].

## ANTIBIOTIC RESISTANCE MECHANISMS

Since the genome sequence of *M. tuberculosis* H37Rv was determined [137], resistance mechanisms against several antibiotics used for treatment of mycobacterial diseases, including those caused by NTM, have been identified at the genome level. Previous studies reported that NTM have natural and acquired antibiotic-resistance mechanisms to several antibiotics and that some of these mechanisms are very similar to those of *M. tuberculosis* [138]. This section will briefly describe studies on natural and acquired resistance to several antibiotics in NTM (Table 2).

### Natural antibiotic resistance

The cell envelope of NTM is a hydrophobic, waxy, impermeable barrier, rich in complex lipids, and thicker than the walls of other bacteria [6]. These features of the cell envelope make NTM naturally resistant to physical and chemical environmental stresses, including antimicrobial agents [139]. In 1994, Jarlier and Nikaido [7] first reported low permeability

as contributing to natural antibiotic resistance and that the high lipid content of the cell envelope was the main factor in this low permeability.

The erythromycin ribosomal methylase (*erm*) gene family contributes to resistance to macrolides, which are among the main antibiotics used for treatment of NTM disease [140]. The *erm* genes confer inducible macrolide resistance, which, in *M. abscessus*, can be ablated by a single nucleotide change at position 28 of the *erm(41)* gene; a functional *erm(41)* gene in *M. abscessus* is an important factor in the inducible resistance of many clinical isolates to macrolides [141]. Not all NTM have functional *erm* genes, but their presence in *M. smegmatis*, *M. abscessus*, *M. bolletii*, and *M. fortuitum* explains the intrinsic macrolide resistance of these species [140,142,143].

Some of the NTM (*M. abscessus*, *M. chelonae*) and *M. tuberculosis* exhibit intrinsic high-level resistance to ethambutol, and this resistance is due to the presence of variant nucleotides within the conserved ethambutol resistance-determining region (ERDR) in *embB* [144,145]. In addition, the quinolone resistance-determining regions (QRDR) in the A and B subunits of DNA gyrase (genes *gyrA* and *gyrB*) were also identified in NTM [146,147]. According to these studies, resistance rates to quinolone differ according to the differences in specific regions of *gyrA* and *gyrB*, which are conserved in the NTM.

The *whiB* gene family includes transcriptional regulators that contribute to intrinsic antibiotic resistance in mycobacteria, including NTM [148,149]. Morris and colleagues [149] found that a null mutant of *M. tuberculosis whiB7* was susceptible to a wide spectrum of antibiotics, and *M. smegmatis* and *M. bovis* mutants lacking *whiB7* genes also showed the same susceptibility pattern as that of the *M. tuberculosis* mutant.

**Table 2.** Summary of types of antibiotic resistance and associated resistance mechanisms in nontuberculous mycobacteria

Type of resistance	Associated gene or cell component	Mechanism	Antibiotic
Natural resistance	Cell envelope	Low permeability	$\beta$ -Lactams
	<i>erm</i> gene	Polymorphisms in <i>erm</i>	Inducible resistance to macrolides
	Ethambutol resistance-determining region	Polymorphisms in <i>embB</i>	Ethambutol
	Quinolone resistance-determining region	Polymorphisms in <i>gyrA</i> and <i>gyrB</i>	Quinolone
	<i>whiB</i> gene	Presence of <i>whiB</i>	Large spectrum of antibiotics
	Efflux pump system	Function of MmpL transporter	Clofazimine, bedaquiline, thiacetazone
Acquired resistance	<i>rpoB</i> gene	Mutation of <i>rpoB</i>	Rifampin
	23S rRNA gene ( <i>rrl</i> )	Mutation of <i>rrl</i>	Macrolides
	16S rRNA gene ( <i>rrs</i> )	Mutation of <i>rrs</i>	Aminoglycosides

*erm*, erythromycin ribosomal methylase; *gyr*, subunits of DNA gyrase; *whiB*, transcriptional regulator whiB; MmpL, mycobacterial membrane protein large; *rpoB*,  $\beta$  subunit of bacterial RNA polymerase; rRNA, ribosomal RNA.

Efflux pump systems are important antibiotic-resistance mechanisms in multidrug resistant (MDR) pathogens, and genes encoding their proteins can be located on chromosomes or plasmids [150,151]. The role of efflux pump systems in antibiotic resistance of NTM has been investigated, and there is some similarity in the mechanism and structure of these efflux pumps with those of *M. tuberculosis* [152-154]. Among the classes of efflux pump systems, mycobacterial membrane protein large (MmpL) and small (MmpS) transporters have emerged as key factors in antibiotic resistance in NTM [155,156]. MmpLs belongs to the resistance-nodulation-cell division permease superfamily [157]. Since Pasca and colleague [158] first reported that the *mmpL7* gene from *M. tuberculosis* conferred resistance to isoniazid when overexpressed in *M. smegmatis*, orthologs of *mmpL* and *mmpS* genes have been characterized and identified in NTM [159]. Recently, several studies have implicated MmpS5/MmpL5-mediated efflux pump systems in antibiotic resistance in NTM. Mutations of the *mmpS5/mmpL5* operon were associated with resistance to bedaquiline and clofazimine in *M. intracellulare* and to thiacetazone in *M. abscessus* [159,160]. Further research may also reveal roles for other efflux pumps of NTM, such as the *mmpL* gene, in antibiotic resistance.

### Acquired antibiotic resistance

The acquisition of resistance by major pathogens is very likely to occur during antibiotic treatment and is mostly caused by mutation of the related genes [161,162]. Due to this acquired resistance, MDR pathogens are rapidly increasing worldwide. The basis for acquired resistance is not always readily identifiable due to interactions among several genes. To date, acquired resistance mechanisms for rifampin, macrolides, and aminoglycosides have been identified in NTM.

Rifampin is the antibiotic primarily used in diseases caused by *M. kansasii* and MAC [163]. However, acquired rifampin resistance has been documented in MAC and *M. kansasii* and is associated with the  $\beta$  subunit of bacterial RNA polymerase (*rpoB*) gene. Mutations within the 81-bp core region of *rpoB* and in other regions of this gene confer rifampin resistance in *M. kansasii* and MAC, and some of these mutations of *rpoB* have previously been described in rifampin-resistant *M. tuberculosis* isolates [163,164].

Macrolides (clarithromycin and erythromycin) are the antibiotics most commonly used in the treatment of NTM, but there are many reports of NTM that are resistant to these antibiotics [1]. A single point mutation at positions 2058 or 2059 (A2058G, A2058C, A2058T, A2059T, A2059C, and A2059G) (*E.*

*coli* numbering) of the 23S rRNA gene (*rrl*) is associated with high-level macrolide resistance in several NTM, including MABC, MAC, *M. chelonae*, *M. fortuitum*, and *M. kansasii* [165-169]. More recently, Mougari and colleagues [170] identified single point mutations of the *rrl* when selecting for macrolide-resistance mutations in MABC *in vitro*. However, they found that acquisition of macrolide resistance by *rrl* mutation was more common in isolates of MABC lacking a functional *erm(41)* gene.

The 2-deoxystreptamine aminoglycosides, including kanamycin, amikacin, gentamicin, and tobramycin, are important drugs for the treatment of MDR NTM and *M. tuberculosis* infection [171]. However, acquired resistance mechanisms for 2-deoxystreptamine aminoglycosides have also been demonstrated in NTM by Prammananan et al. [172] and Wallace et al. [173]. According to these studies, *M. abscessus* showed a very high resistance rate to kanamycin, amikacin, and tobramycin due to a single mutation at position 1408 (A1408G) (*E. coli* numbering) of the 16S rRNA gene (*rrs*). In addition, mutations of positions 1406, 1409, and 1491 of *rrs* (T1406A, C1409T, and G1491T) as well as position 1408 were associated with aminoglycoside resistance [174]. Other researchers have also reported that *rrs* mutations are associated with aminoglycoside resistance in mycobacteria other than *M. abscessus* [175-177].

## CONCLUSION

The incidence and prevalence of NTM lung disease are increasing worldwide, and NTM have natural and acquired resistance mechanisms to several antibiotics used in treatment. NTM infection depends on sufficient environmental exposure, host susceptibility factors, and mycobacterial virulence factors. Further knowledge of the ecology and transmission routes of these organisms, as well as host-pathogen interactions, will enable better interventions to both prevent and treat NTM disease.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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