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Diagnostics of non-tuberculous mycobacteria

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1 Introduction.

General introduction.

Mycobacteria belong to the family *Mycobacteriaceae* and are members of the CMN group (*Corynebacteria, Mycobacteria and Nocardia*). The family *Mycobacteriaceae* are Gram-positive, nonmotile, catalase-positive, have a rodlike to filamentous morphology and can be pleomorphic. As a group, they produce characteristic long chain fatty acids termed mycolic acids. Mycobacteria are acid-fast rods of variable appearance, approximately 0.2-0.6 by 1-10 micrometer.

The genus *Mycobacterium* consists of 127 species (excluding subspecies) according to the latest approved list of bacterial species *(1)*. Mycobacteria other than *Mycobacterium tuberculosis* are commonly referred to as atypical or non-tuberculous mycobacteria (NTM). Two of these cause disease in normal hosts and are thus primary pathogenic: *M. leprae, M. ulcerans*. They are often not regarded as NTM. The remaining species are considered nonpathogenic or opportunistic pathogens and cause disease when host-defences are compromised. Mycobacteria can be arranged into four groups according to the Runyon classification:

- Group 1 – Photochromogens: slow growers and form pigment when exposed to light (eg, *M. kansasii, M. marinum, M. simiae*)
- Group 2 – Scotochromogens: slow growers and form pigment in the dark (eg, *M. scrofulaceum, M. szulgai, M. gordonae*)
- Group 3 – Nonphotochromogens: slow growers and not pigmented (eg, *M. malmoense, M. xenopi, M. avium*-complex, *M. ulcerans, M. haemophilum*)
- Group 4 - Rapid growers (eg, *M. fortuitum, M. chelonae, M. abscessus*)

Most slow-growing species have been associated with disease in humans while only few species of group 4 (the ones mentioned above) are disease associated.

Since the advent of AIDS and the application of recent developments in molecular biology for the detection and identification of NTM, NTM infections are increasingly detected. This in its turn created a higher awareness for mycobacterial involvement in a variety of clinical conditions and NTM diseases have been increasingly recognized in immunocompetent patients as well.

Taxonomy of the genus Mycobacterium.

Unidentified species are constantly being discovered and mycobacterial taxonomy is continuously changing. The species belonging to a species group (sometimes referred to as a species-complex) can be very different in virulence or pathogenesis.
Several previously considered species appear to consist of several closely related species, as biochemical and mainly genetic analyses have demonstrated in, for instance, the *M. tuberculosis*-complex. The complex species can be identified using differences in size of genomic sequences: Region of Difference1 (RD1), RD2, RD4, RD9 and RD12, analysed in PCR *(2)*. This complex now consists of *M. tuberculosis, M. africanum, M. microti* and *M. bovis*, the latter of which has been further differentiated into *M. bovis*, *M. caprae, M. pinnipedii* and *M. canetii,* all named after their original host *(2)*. A similar phenomenon can be found in the *M. avium*-complex. Originally, one species, but meanwhile divided into the species *M. avium*, *M. intracellulare* and *M. scrofulaceum.* Previous serovars belonging to this complex have been re-arranged *(3, 4, 5)* and several subspecies of *M. avium* have been identified: *M. avium subsp. paratuberculosis*, *subsp. silvaticum* and *subsp. avium*. This identification was based on molecular and biochemical criteria, that is High Performance Liquid Chromatography (HPLC) of mycolic acids and sequencing of, for instance, Internal Transcribed ribosomal Regions (ITS). *M. avium subsp. avium*, has recently been divided in *subsp. avium* and *subsp. hominissuis* using Restriction Fragment Length Polymorphism (RFLP) of a insertion sequence *IS*1245 *(6)*. The majority of human infections is caused by *M. hominissuis (6)*.

The identification of a new species was conventionally based on the description of the Runyon classification, the biochemical properties of the strain(s) and the degree of DNA-DNA hybridization. With the growing amount of species and subspecies with various biochemical properties, new methods were developed and this created a new standard for species differentiation *(7*). For the acceptance of a new species, the old and new ways of identification are all included: biochemical characteristics, growth and pigmentation characteristics, HPLC analysis and a unique genetic composition determined by the sequence of genes that allow species differentiation, such as the 16S rRNA gene, the *hsp65* gene and the ITS region, as applied and subsequently published in the International Journal of systematic Bacteriology *(1, 8, 9).* Sequencing of at least two targets as mentioned above must be included, but the choice of targets is not specified. From 1990 to 1999, 28 new species have been recognized *(9)* and from 2000 to September 2007, 41 more species have been identified (Table 1). Of the latter 41, at least 26 were the cause of disease in humans, and at least six new species of clinical origin were rapid growers not belonging to one of the known clinical species groups. This illustrates the present discovery rate within mycobacterial taxonomy.

Table 1: New NTM determined from January 2000 to September 2007.

year	species name	source	runyon group	additional info
2000	*M. botniense*	water	2	*M. xenopi*-like
2000	*M. kubicae*	human	2	between slow / rapid growers
2000	*M. septicum*	human	4	
2000	*M. elephantis*	elephant	4	
2001	*M. heckeshornense*	human	2	*M. xenopi*-like
2001	*M. doricum*	human	2	
2001	*M. immunogenum*	human	4	*M. abscessus* group
2001	*M. frederiksbergense*	contaminated soil	4	
2002	*M. palustre*	human / other	2	
2002	*M. lacus*	human	3	
2002	*M. vanbaalenii*	contaminated soil	4	
2002	*M. holsaticum*	human	4	
2003	*M. pinnipedii*	seal		*M. tuberculosis*-complex
2003	*M. shottsii*	striped bass	3	no growth >30 ^{0}C
2003	*M. montefiorense*	eels	3	no growth >30 ^{0}C
2004	*M. saskatchewanense*	human	2	
2004	*M. parascrofulaceum*	human	2	
2004	*M. parmense*	human	2	
2004	*M. nebraskense*	human	2	
2004	*M. chimaera*	human	3	MAC group
2004	*M. psychrotolerans*	pond water	4	
2004	*M. canariasense*	human	4	
2004	*M. cosmeticum*	human	4	
2004	*M. pyrenivorans*	contaminated soil	unknown	
2004	*M. massiliense*	human	4	*M. abscessus* group
2005	*M. florentinum*	human	unknown	slow growing pigment forming
2005	*M. pseudoshottsii*	striped bass	1	
2006	*M. arupense*	human	3	rapid growing at 30 ^{0}C
2006	*M. phocaicum*	human	4	
2006	*M. neworleansense*	unknown	4	*M. fortuitum* group
2006	*M. houstonense*	unknown	4	*M. fortuitum* group
2006	*M. aubagnense*	human	4	*M. mucogenicum* group
2006	*M. bolletii*	human	4	*M. abscessus* group
2006	*M. boenickei*	unknown	4	*M. fortuitum* group
2006	*M. conceptionense*	human	4	*M. fortuitum* group
2006	*M. fluoranthenivorans*	soil	unknown	
2006	*M. kumamotonense*	human	3	*M. terrae* group
2006	*M. colombiense*	human	3	MAC group
2006	*M. monacense*	human	4	
2006	*M. brisbanense*	unknown	4	*M. fortuitum* group
2007	*M. seoulense*	human	2	

Disease caused by NTM.

The most common sites where mycobacterial disease occurs are the lungs, the lymph nodes and skin. However, as *M. tuberculosis* is mostly known to cause the well-established pulmonary manifestation but is capable of infecting virtually all tissue types, the NTM species follow the same behaviour: the range of clinical manifestations is extensive *(10)*.

Pulmonary infections.

Pulmonary involvement is most common in immunocompromised patients. *M. avium* or *M. kansasii* infection are the predominant species known in AIDS patients *(11)*, but nowadays *M. avium* is frequently encountered in immunocompetent patients, probably due to the improved diagnostic tools and clinical awareness. While NTM infection in immuno competent patients is increasingly common, a high bacterial burden or a damaged epithelial are usually cofactors for infection in these patients *(12)*.

Studies at the reference laboratories in Australia revealed 80% of clinical NTM isolates to be derived from pulmonary sources. However, as stated by the reference laboratories, the significance of an isolate is often doubtful when isolated from pulmonary sources and in Australia only 10% of all pulmonary isolates are associated with disease (clinically significant). In contrast, almost all NTM isolates (91-98%) from lymphatic, bone or soft tissue (skin and joint) origin were clinically significant. Because many NTM species are ubiquitous, the detection of NTM in clinical materials is not per sé proof of the identification of the cause of disease. This is especially true for the detection in non-sterile materials. The criteria for the clinical significance of positive NTM diagnostics have been described by the American Thoracic Society (ATS) *(13)*.

Still, pulmonary infection with NTM can take several forms which are evidently associated with disease *(13, 14)*. The first, or classical, form is radiographically indistinguishable from tuberculosis. It is characterized by nodular opacities in the apices, cavitation and/or apical pleural thickening *(12)*. 80%-90% of the patients are elderly Caucasian males and it is often found secondary to other lung disease. The most common species are *M. avium, M. kansasii* and *M. malmoense (12)*. Risk factors include smoking, alcoholism, cardiovascular disease, chronic liver disease, and previous gastrectomy. Symptoms include coughing (60-100%), weight loss, fever (10-13%), weakness, and hemoptysis (15-25%), but are often mild or completely absent *(15)*. The second form is non-classical, does not resemble tuberculosis and *M. avium* is primarily the species involved. This infection is characterized by an interstitial and/or nodular pattern instead of a cavitary pattern involving mostly the lingula or middle lobe of the right lung. Risk factors are poorly understood. It is not necessarily related to smoking or any underlying chronic lung disease and is found most often in middle-aged or elderly women (Lady Windermere syndrome) *(16, 17, 15)*. The third form of mycobacterial pulmonary disease is the “hot-tub lung” affecting middle-aged patients, male and female. It is mostly caused by species belonging to the *M. avium*-complex and is often recognized in metal-fluid workers and indoor swimming pool staff. Primary differences to other forms of pulmonary mycobacterial disease are the diffuse nodular presentation and the acute

manifestation instead of chronic manifestation *(18, 19, 20)*. Already the most common species involved in pulmonary tract infections in immunocompromised patients, the incidence proportion of *M. avium* in immunocompetent patients is increasing rapidly *(21)*.

Skin infections.

Cutaneous NTM infections result from external inoculation at sites of trauma, the spread of a deeper infection from the joints or other tissues, or haematogenous spread of a disseminated infection. There are a few species-specific infections (fish tank or swimming pool granuloma, due to *M. marinum*, Buruli ulcer, caused by *M. ulcerans* and Leprosy, caused by *M. leprae*). Most species, however, produce a nonspecific clinical picture, like *M. haemophilum* or *M. abscessus* and are mostly encountered in industrialised countries. Lesions occur in various forms as suppurative nodules, ulcers, abscesses, sporotrichoid lesions, folliculitis, furunculosis and indurated plaques. In immunocompetent patients the infection is normally localized, superficial and limited to the extremities. In immunosuppressed patients the number of lesions is often multiple and cutaneous involvement is often accompanied by disseminated disease *(22)*. Abscesses and ulceration are also more frequently observed in immunosuppressed patients.

The risk factors for NTM infection include: 1) HIV infection, lymphoma, leukemia or immunosuppressive therapy. Immunosuppression is responsible for the increase of cutaneous infections by a large variety of species, particularly in industrialized countries. 2) The natural environment is directly responsible for the emergence of cutaneous infections caused by a small number of species including *M. avium* and *M. marinum* in Europe and North America, and *M. ulcerans* in the tropics. 3) The medical environment when sterilization is inadequate is also not uncommonly responsible *(23).*

Different histopathological patterns can be noted in biopsy specimens from cutaneous nontuberculous mycobacterial infections. The evolution of the disease and the immunologic status of the host may explain this spectrum of morphological changes. Tuberculoid, palisading and sarcoid-like granulomas, a diffuse infiltrate of histiocytic foamy cells, acute and chronic panniculitis, non-specific chronic inflammation, cutaneous abscesses, suppurative granulomas and necrotizing folliculitis can be detected. Suppurative granulomas are the most characteristic feature in skin biopsy specimens from cutaneous NTM infections. A marked granulomatous inflammatory reaction is more common in immunocompetent than in immunosuppressed patients *(24)*. Both sexes are equally affected but males predominate in *M. marinum* infection and females predominate in rapid growers. All ages can be affected, but most cases involve middle-aged people. Cervical lymphadenitis and cutaneous abscesses are the common manifestations of rapid-grower infections. Hyperkeratotic verrucous plaques (tuberculosis verrucosa cutis-like) and sporotrichoid lesions are the common manifestations of slow-grower infection *(25)*.

Lymphadenitis.

NTM lymphadenitis is seen in immunocompromised patients, but is mostly known as the most common manifestation of NTM disease in immunocompetent patients and usually

affects children under the age of 12 as chronic cervicofacial lymphadenitis. It is more common in industrialized countries and is suggested to be a “prosperity disease” *(26)*. This age group may also be more susceptible because of a lack of a fully-developed immune system *(27)*.

Occurrence of lymphadenitis in immunocompromised patients is often accompanied by disseminated mycobacterial disease *(28)* and affects lymph nodes at different body sites. In lymphadenitis in “healthy” children, involvement of only one single lymph node is common except in *M. haemophilum* infection, where the involvement of multiple lymph nodes is more common (56%) *(29)*. Involvement of submandibular lymph nodes are seen in 75% of the patients, while preauricular or periparotid sites of infection account for 12% to 25% of NTM adenitis cases. The clinical features include non-tender enlargement of the lymph node and violaceous skin discoloration of the overlying skin. After several weeks to months, caseous necrosis develops and when untreated, spontaneous drainage can occur leaving scars. The ports of entry are the pharyngeal mucosa, tonsils, conjunctiva, gingiva, and salivary glands. Ingestion of contaminated soil or water is speculated to be the source of infection. In salivary gland infection, the possibility of retrograde passage of the mycobacteria along the duct exists, thumb sucking being a possible risk factor in children *(30, 31)*.

Studies reported an annual incidence varying from 1,21 to 1.78 cases per 100.000 children which is increasing *(32, 33, 34, 35)*. In the Netherlands, the estimated annual incidence of NTM cervicofacial infections is 0.77 per 100.000 children *(36)*. Earlier publications describe a higher incidence of cervical lymphadenitis in winter and spring *(37, 38),* but this is contradicted by Lindeboom et al who did not observe a seasonal difference in the Netherlands: autumn (29% of patients), winter (25%), spring (25%), and summer (21%) *(29).*

The number of mycobacterial species is increasing and several newly identified species have first been encountered in lymphadenitis patients *(39, 40)*. However, approximately 70% of the cervicofacial lymphadenitis cases are caused by *M. avium (41)*. Other frequently involved species depend on the geographical distribution of mycobacterial species. In India *M. scrofulaceum* is commonly involved, and it used to be the most prevalent species in the United States. In Israel, Australia and The Netherlands, *M. haemophilum* is the second most common species *(14, 32, 42, this thesis chapters 3 and 4)*. In the rest of Europe this is *M. malmoense (12)*. The switch in species prevalence is thought to be caused by variability in their presence in natural sources *(21, 43)*.

<u>Disseminated disease.</u>

Disseminated NTM infection in HIV or otherwise immunodeficient patients appears to originate from a primary infection of either the skin or respiratory or gastrointestinal tracts.

These infections may involve any organ, but most commonly occur in the lungs, liver, spleen, lymph nodes or bone marrow. Common symptoms include prolonged fevers (often accompanied by night sweats), weight loss and occasional abdominal pain or diarrhea. This disease is most commonly seen in patients with less than 50 CD4 cells *(13)*. The primary *Mycobacterium* species associated with disseminated infections in HIV infected patients is

M. avium. However, *M. chelonae, M. abscessus, M. kansasii, M. haemophilum, M. genavense* and sporadically *M. scrofulaceum* have also been implicated *(44)*.

Skeletal infections.

NTM (usually slow-growing species) can cause skeletal infections as well, which often affect the synovium or osteoarticular components of the extremities, but may occur at other skeletal sites (i.e. osteomyelitis), particularly when there is underlying immunosuppression. Monoarthritis is the most common, but polyarthritis has been reported as well *(45, 46, 47, 48, 49)*.

Gastrointestinal infections.

Both slow-growing and rapid-growing species, have been isolated from intestinal specimens from patients with Inflammatory bowel disease (Crohn's disease), ulcerative colitis, and non-inflammatory bowel diseases *(50)*. It is still controversial which role mycobacterial infections have in the etiology of Crohn's disease. Several studies suggested *M. avium* spp *paratuberculosis* (MAP) as the primary cause in the etiology of Crohn's disease and reported positive cultures for MAP and PCRs or high specific immune responses *(51, 52, 53, 54, 55)*. A significant correlation between Crohn's disease and MAP has even been established *(56*), but the exact role of the mycobacterium remains to be defined *(56)*. One of the popular theories on Crohn's disease is the autoimmune theory which suggests that the disease results from inappropriate ongoing activation of the mucosal immune system driven by the presence of normal luminal flora *(57)*. This would place the infectious agent in a secondary position but, nevertheless, in an active role.

Foreign body related and nosocomial infections.

Keratitis is another manifestation of NTM disease. This eye infection, usually caused by rapid growers, can cause significant damage when not treated properly. The typical clinical features consist of irregular corneal infiltrates with radiating projections, indistinct fluffy lesion margins, satellite lesions and associated epithelial defect *(58)*. More than 150 cases have been reported to date, the majority of which in Asian countries. The major risk factor is injury to and the presence of foreign bodies in the cornea, frequent use of lens fluid and surgical trauma *(58, 59, 60, 61)*. Infection following laser-assisted in situ keratomileusis (LASIK) has been more commonly described in recent years *(62, 63, 64, 65)*.

Other infections with NTM subsequent to surgical or other invasive medical procedures have been reported as well, predominantly caused by rapid-growing species. *(66, 67, 68, 69)*. Postsurgical inflammatory complications with NTM present a difficult challenge because of the resistant nature of mycobacteria against disinfectants used in the cleaning of hospital equipment. NTM have been encountered in hospital water supplies *(70, 71)* and have in some cases been linked to pseudo-outbreaks *(72, 73)*.

Table 2: clinical manifestations and the most commonly encountered NTM species. Adapted from Wagner et al. 2004) *(12, 14, 74, RIVM personal correspondence)*

clinical manifestation	**common species**	**less common species**	
pulmonary disease	*M. avium*-complex	*M. simiae*	*M. asiaticum*
	M. kansasii	*M. szulgai*	*M. shimodii*
	M. abscessus	*M. fortuitum*	*M. smegmatis*
	M. chelonae	*M. celatum*	*M. haemophilum*
	M. xenopi	*M. gordonae*	
	M. malmoense		
lymphadenitis	*M. avium*-complex	*M. fortuitum*	*M. interjectum*
	M. malmoense	*M. kansasii*	*M. heidelbergense*
	M. haemophilum	*M. abscessus*	*M. scrofulaceum*
		M. chelonae	*M. bohemicum*
		M. lentiflavum	
skin and soft-tissue disease	*M. ulcerans*	*M. kansasii*	
	M. marinum	*M. malmoense*	
	M. haemophilum	*M. chelonae*	
	M. abscessus	*M. smegmatis*	
	M. avium-complex	*M. fortuitum*	
disseminated disease	*M. avium*-complex	*M. celatum*	*M. scrofulaceum*
	M. kansasii	*M. conspicuum*	*M. abscessus*
	M. haemophilum	*M. malmoense*	*M. simiae*
	M. fortuitum	*M. genavense*	
	M. chelonae	*M. xenopi*	
skeletal infection	*M. avium*-complex	*M. chelonae*	*M. marinum*
	M. abscessus	*M. kansasii*	*M. smegmatis*
	M. fortuitum	*M. scrofulaceum*	*M. nonchromogenicum*
		M. haemophilum	*M. malmoense*
		M. xenopi	*M. szulgai*
Gastrointestinal infection	*M. avium*-complex	*M. mucogenicum*	
		M. kansasii	
foreign body-related infections and nosocomial infections	*M. fortuitum*	*M. mucogenicum*	*M. smegmatis*
	M. abscessus	*M. neoaurum*	*M. avium*-complex
	M. chelonae	*M. aurum*	
		M. gordonae	
		M. simiae	

Natural reservoirs.

NTM are saprophytes and ubiquitous and can exist in soil, dust, food (eggs, raw milk, vegetables) and water *(43, 75)*. Animal reservoirs are also proposed to be involved in the etiology of human disease *(46)*: *M. avium* has been recovered from the lymph nodes of swine and domestic fowl; *M. genavense, M. fortuitum,* and *M. avium* from birds; *M. chelonae* from fish and frogs *(12) M. haemophilum* from cockroaches *(76)* and *M. ulcerans* from mosquitoes *(77)*. Many species have been isolated from natural water and drinking water systems and appear highly capable of forming biofilms, are able to sustain disinfecting treatment and are present in aerosols *(78).*

Pathogenesis and host defences.

Mycobacteria are thermoresistant, endure most disinfectants and have the ability to form biofilms. This is due to their thick acid-fast cell wall. No human-to–human transmission has been recognised for mycobacteria other than *M. tuberculosis* and *M. leprae*. Humans are thought to get infected through the inhalation of aerosols (showering, swimming) or direct contact with the bacteria by skin or mucosa with affected integrity *(79)*. Pulmonary infections can occur in patients with impaired ventilation systems but without specific immunity problems. Children with cervical lymphadenitis also are considered healthy. Therefore, the mycobacteria need to possess ingenious mechanisms to evade the host defences.

Mycobacteria have the capacity to thrive inside macrophages. As part of the immune system, macrophages are capable of destroying a wide variety of bacterial pathogens. Mycobacteria, however, are one of the few types of bacteria that are not only able to survive the antibacterial effects of macrophages, but actually grow and multiply inside them.

Considerable research has been done to try and understand how mycobacteria flourish in - what is thought to be- the hostile intracellular environment of macrophages. Two properties of mycobacteria explain their resistance to being killed by macrophages: The first is the cord factor that can neutralize the antibacterial chemicals produced inside macrophages and inactivate mitochondrial membranes of phagocyts *(80)*. Cord factor (trehalose 6, 6'-dimycolate) is a glycolipid in the cell wall of mycobacteria. They are mostly known as the molecules responsible for the serpentine cord-forming growth characteristics of *M. tuberculosis*, but comparable growth phenomena are encountered in non-tuberculous species caused by variable forms of cord factor in the cell wall as well. The glycolipid is widely distributed as a potent immunomodifier among non-tuberculous mycobacteria and related micro-organisms such as *Corynebacterium (81)*.

The second is the chemically unique mycobacterial cell wall that is resistant to destruction or penetration. The cell wall of mycobacteria is composed of a mixture of lipids and polysaccharides. The lipids in the cell wall inhibit the migration of macrophages, have the capacity to disrupt phagosomal membranes of alveolar macrophages and disrupt normal

cytokine signalling that is responsible for the ineffective cell-mediated immune respons *(82, 83, 84, 85).*

Pathological properties of NTM might vary greatly between species. This is illustrated by three closely related species, *M. marinum, M. ulcerans* and *M. haemophilum*: All three cause necrotizing skin disease, are taxonomically related *(86)* and share common reservoirs (stagnant or slow-flowing water) *(87)*, but pathogenic differences are noted. *M. ulcerans* is highly pathogenic for humans and causes specific large necrotic ulcers, while *M. marinum* and *M. haemophilum* are responsible for mostly self-limiting and slowly progressing granulomatous lesions *(87)*. *M. marinum* causes disease- but seldom death- in fish, while *M. haemophilum* infection in fish results readily in death *(88)*. Infections with *M. marinum* and *M. ulcerans* are almost always restricted to the skin, but *M. haemophilum* often causes disease in deeper tissues. This is in contrast with the in vitro growth charachteristics of the three species: *M. haemophilum* has great difficulties to grow at higher temperatures, *M. marinum* and *M. ulcerans* grow at normal culture temperatures of 35-37˚C but faster at lower temperatures (30-32 ˚C). Deep tissue infections would logically be restricted to species able to grow at higher temperatures.

Iron uptake of mycobacterial species also differs. Because mycobacteria require iron in pathogenesis and the iron levels in inflammation processes are elevated, the strong cellular immune response of the host is induced by mycobacteria *(89, 90)*. *M. haemophilum* however, is the only species that requires iron-additives added to the culture and this demonstrates the differences in iron-management between NTM.

Pathogenic properties of NTM can be transferred between species. An example of a potentially hazardous change is the transfer of the toxin produced by *M. ulcerans*. This mycolactone causes the destructing properties of this pathogenic species. Previously, only *M. ulcerans* was known to harbour a virulence plasmid with the gene for mycolactone *(91)*. Recently, in strains of *M. marinum* and *M. pseudoshottsii,* responsible for death in fish, a mycolactone variant has been identified. This gene is suspected to have been spread by horizontal transfer *(92, 93)*.

Secundary to the pathogenic properties of the mycobacteria themselves, impaired host defences are thought to be responsible for the susceptibility of healthy patients to NTM because, due to the environmental presence, humans are continuously exposed to the bacteria in low levels (50-500 bacilli per day) *(43, 85)*. Acquired human resistance is cell-mediated, antibodies do not have a protective role. T lymphocytes lyse infected macrophages directly or activate them via soluble mediators to destroy intracellular bacteria. Mutations in the genes responsible for this mechanism create resistance disorders. Mutations in the interferon-gamma receptor ligand-binding chain (IFN gamma R1), interferon-gamma receptor signalling chain (IFN gamma R2), Signal Transducer and Activator of Transcription-1 (STAT-1), interleukin-12 p40 subunit (IL-12 p40), and interleukin-12 receptor beta 1 chain (IL-12R beta 1) genes have all been identified as predisposing factors for NTM infections *(94, 85)*. Dominant or recessive alleles causing complete or partial

cellular defects have been found to define nine different inheritable disorders for the susceptibility of patients for opportunistic pathogens *(95, 96, 97)*.

Treatment of NTM.

Guidelines for diagnosis and treatment have been produced for NTM by the British Thoracic Society (BTS) and the American Thoracic Society (ATS) *(13, 98)*. Diagnosis is addressed in chapter 2 of this thesis. Criteria for the treatment of NTM infection is based on the species involved, the immune characteristics of the patient and the clinical manifestation of the infection.

Two basic rules apply to mycobacterial disease: 1) For all mycobacterial infections long therapy is necessary (2-24 months), which is a direct consequence of the slow-growing properties of the genus. 2) Mycobacteria rapidly obtain resistance to the most common antibiotics and therefore dual or multiple combinations of antibiotic groups are common regimens *(99, 100).*

Side effects to antituberculous drugs and NTM regimens are common due to the toxicity of the agents and include hepatitis, cutaneous reactions, gastrointestinal intolerance, haematological reactions and renal failure *(101, 102)*. This results in modification or discontinuing of the therapy *(103)*. The only alternative for antibiotic regimens is surgical excision in some cases. Treatment with steroids is not a safe alternative therapy *(104)*. Mycobacteria are intrinsically resistant to most common antibiotics and while *M. tuberculosis* is resistant to macrolides, NTM are often resistant to first-line antituberculous drugs which emphasises the importance of species identification in mycobacterial disease *(99)*.

NTM infection with slow growing species in immunocompetent patients is often treated by a three-component or dual therapy of oral clarithromycin, rifabutin, ciprofloxacin, rifampicin and ethambutol *(105)*. The normal duration of the therapy is 4-24 months depending on the clinical manifestation (e.g. 4-6 months in lymphadenitis, 24 months in bone-infections) *(13, 98)*. In children with lymphadenitis in the Netherlands, surgical excision of the affected lymph nodes was the treatment with the highest cure rate. A combination of clarithromycin and rifabutin appeared less effective in these patients *(106)*. In other countries, the same treatment for lymphadenitis is recommended *(107, 108)*. For cutaneous or localised lung disease, surgical treatment is preferred in many cases as well, taking scarring and other complications into account. However, for *M. marinum* infection medical therapy is the treatment of choice *(25)*.

Treatment in immunocompromised patients is often given for a longer duration than in immunocompetent patients: most treatments are administered for many years. Also, sometimes profylaxis is given in AIDS patients: lifelong azithromycin, clarithromycin or azithromycin + rifabutin *(13)*. Drawbacks of antimycobacterial therapy in AIDS patients are the interactions with antiretroviral therapy, which need to be closely monitored *(13, 98)*.

References: 1 Introduction.

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