



Universiteit
Leiden
The Netherlands

Diagnostics of non-tuberculous mycobacteria

Bruijnesteijn van Coppenraet, L.E.S.

Citation

Bruijnesteijn van Coppenraet, L. E. S. (2009, March 5). *Diagnostics of non-tuberculous mycobacteria*. Retrieved from <https://hdl.handle.net/1887/13665>

Version: Corrected 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/1887/13665>

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

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. goodii*)
- 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. canettii*, 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 *IS1245* (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	<i>M. botniense</i>	water	2	<i>M. xenopi</i> -like
2000	<i>M. kubicae</i>	human	2	between slow / rapid growers
2000	<i>M. septicum</i>	human	4	
2000	<i>M. elephantis</i>	elephant	4	
2001	<i>M. heckeshornense</i>	human	2	<i>M. xenopi</i> -like
2001	<i>M. doricum</i>	human	2	
2001	<i>M. immunogenum</i>	human	4	<i>M. abscessus</i> group
2001	<i>M. frederiksbergense</i>	contaminated soil	4	
2002	<i>M. palustre</i>	human / other	2	
2002	<i>M. lacus</i>	human	3	
2002	<i>M. vanbaalenii</i>	contaminated soil	4	
2002	<i>M. holsaticum</i>	human	4	
2003	<i>M. pinnipedii</i>	seal		<i>M. tuberculosis</i> -complex
2003	<i>M. shottsii</i>	striped bass	3	no growth >30 °C
2003	<i>M. montefiorensis</i>	eels	3	no growth >30 °C
2004	<i>M. saskatchewanense</i>	human	2	
2004	<i>M. parascrofulaceum</i>	human	2	
2004	<i>M. parmense</i>	human	2	
2004	<i>M. nebraskense</i>	human	2	
2004	<i>M. chimaera</i>	human	3	MAC group
2004	<i>M. psychrotolerans</i>	pond water	4	
2004	<i>M. canariensis</i>	human	4	
2004	<i>M. cosmeticum</i>	human	4	
2004	<i>M. pyrenivorans</i>	contaminated soil	unknown	
2004	<i>M. massiliense</i>	human	4	<i>M. abscessus</i> group
2005	<i>M. florentinum</i>	human	unknown	slow growing pigment forming
2005	<i>M. pseudoshottsii</i>	striped bass	1	
2006	<i>M. arupense</i>	human	3	rapid growing at 30 °C
2006	<i>M. phocaicum</i>	human	4	
2006	<i>M. neworleansense</i>	unknown	4	<i>M. fortuitum</i> group
2006	<i>M. houstonense</i>	unknown	4	<i>M. fortuitum</i> group
2006	<i>M. aubagnense</i>	human	4	<i>M. mucogenicum</i> group
2006	<i>M. bolletii</i>	human	4	<i>M. abscessus</i> group
2006	<i>M. boenickei</i>	unknown	4	<i>M. fortuitum</i> group
2006	<i>M. conceptionense</i>	human	4	<i>M. fortuitum</i> group
2006	<i>M. fluoranthenvivans</i>	soil	unknown	
2006	<i>M. kumamotoense</i>	human	3	<i>M. terrae</i> group
2006	<i>M. colombiense</i>	human	3	MAC group
2006	<i>M. monacense</i>	human	4	
2006	<i>M. brisbanense</i>	unknown	4	<i>M. fortuitum</i> group
2007	<i>M. seoulense</i>	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 immunocompetent patients is increasingly common, a high bacterial burden or a damaged epithelium 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 se 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).

Disseminated disease.

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

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 phagocytes (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 responses (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 characteristics 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).

Secondary 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 prophylaxis 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.

1. Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures) Website DSMZ: http://www.dsmz.de/microorganisms/bacterial_nomenclature.php
2. Warren RM, van Pittius NC, Barnard M, Hesselting A, Engelke E, de Kock M, Gutierrez MC, Chege GK, Victor TC, Hoal EG, van Helden PD. Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic regions of difference. *Int J Tuberc Lung Dis.* 2006;10:818-22.
3. Butler WR, Thibert L, Kilburn JO. Identification of *Mycobacterium avium* complex strains and some similar species by high-performance liquid chromatography. *J Clin Microbiol.* 1992;30:2698-704.
4. LG Wayne, Good RC, Tsang A, Butler R, Dawson D, Groothuis D, Gross W, Hawkins J, Kilburn J, Kubin M. 1993. Serovar determination and molecular taxonomic correlation in *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*: a cooperative study of the International Working Group on Mycobacterial Taxonomy. *Int J Syst Bacteriol.* 1993;43:482-489.
5. Thorel MF, Krichevsky M, Levy-Frebault VV. Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. nov., *Mycobacterium avium* subsp. *paratuberculosis* subsp. nov., and *Mycobacterium avium* subsp. *silvaticum* subsp. nov. *Int J Syst Bacteriol.* 1990;40:254-60.
6. Mijs W, de Haas P, Rossau R, Van der Laan T, Rigouts L, Portaels F, van Soolingen D. Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp. *avium* for bird-type isolates and '*M. avium* subsp. *hominissuis*' for the human/porcine type of *M. avium*. *Int J Syst Evol Microbiol.* 2002;52:1505-18.
7. Rogall T, Wolters J, Flohr T, Bottger EC. Towards a phylogeny and definition of species at the molecular level within the genus *Mycobacterium*. *Int J Syst Bacteriol.* 1990;40:323-30.
8. The International Journal of Systematic and Evolutionary Microbiology (IJSEM) website: <http://ijs.sgmjournals.org>.
9. Hale YM, Pfyffer E, Salfinger M. Laboratory Diagnosis of Mycobacterial Infections: New Tools and Lessons Learned. *Clin Infect Dis.* 2001;33:834-846.
10. Ellis SM. The spectrum of tuberculosis and non-tuberculous mycobacterial infection. *Eur Radiol.* 2004;14 (Suppl 3):E34-42.
11. Coulter C, Robson J. Skin and soft tissue infections due to non-tuberculous mycobacteria. *Microbiol Australia.* 2004;25:10-14.
12. Wagner D, Young LS. Nontuberculous mycobacterial infections: a clinical review. *Infection.* 2004;32:257-70.
13. American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA). An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases. *Am J Respir Crit Care Med.* 2007;175:367-416.
14. Haverkort F; Australian Mycobacterium Reference Laboratory Network; Special Interest Group in Mycobacteria within the Australian Society for Microbiology. National atypical mycobacteria survey, 2000. *Commun Dis Intell.* 2003;27:180-9.
15. Sadow CA. Pulmonary Mycobacterial (*Avium* Complex) Infection. 2002. website: <http://brighamrad.harvard.edu/Cases/bwh/hcache/332/full.html>
16. Dhillon SS, Watanakunakorn C. Lady Windermere syndrome: middle lobe bronchiectasis and *Mycobacterium avium* complex infection due to voluntary cough suppression. *Clin Infect Dis.* 2000;30:572-5.
17. Gardner TJ. Evaluation of *Mycobacterium avium* complex lung disease in women. 2004. Dissertation. ISBN 0-496-01687-3.
18. Khor A, Leslie KO, Tazelaar HD, Helters RA, Colby TV. Diffuse pulmonary disease caused by nontuberculous mycobacteria in immunocompetent people (hot tub lung). *Am J Clin Pathol.* 2001;115:755-62.

19. Hanak V, Kalra S, Aksamit TR, Hartman TE, Tazelaar HD, Ryu JH. Hot tub lung: presenting features and clinical course of 21 patients. *Respir Med.* 2006;100:610-5.
20. Marchetti N, Criner K, Criner GJ. Characterization of functional, radiologic and lung function recovery post-treatment of hot tub lung. A case report and review of the literature. *Lung.* 2004;182:271-7.
21. Henry MT, Inamdar L, O'Riordain D, Schweiger M, Watson JP. Nontuberculous mycobacteria in non-HIV patients: epidemiology, treatment and response. *Eur Respir J.* 2004;23:741-6.
22. Bartralot R, Garcia-Patos V, Sitjas D, Rodriguez-Cano L, Mollet J, Martin-Casabona N, Coll P, Castells A, Pujol RM. Clinical patterns of cutaneous nontuberculous mycobacterial infections. *Br J Dermatol.* 2005;152:727-34.
23. Gbery IP, Djeha D, Yobouet P, Aka B, Kanga JM. Atypical mycobacterial skin infections. *Sante.* 1996;6:317-22.
24. Bartralot R, Pujol RM, Garcia-Patos V, Sitjas D, Martin-Casabona N, Coll P, Alomar A, Castells A. Cutaneous infections due to nontuberculous mycobacteria: histopathological review of 28 cases. Comparative study between lesions observed in immunosuppressed patients and normal hosts. *J Cutan Pathol.* 2000;27:124-9.
25. Mahaisavariya P, Chaiprasert A, Khemngern S, Manonukul J, Gengviniij N, Ubol PN, Pinitugsorn S. Nontuberculous mycobacterial skin infections: clinical and bacteriological studies. *J Med Assoc Thai.* 2003;86:52-60.
26. Eriksson M, Bennet R, Danielsson N. Non-tuberculous mycobacterial lymphadenitis in healthy children: another "lifestyle disease"? *Acta Paediatr.* 2001;90:1340-2.
27. Olson NR. Nontuberculous mycobacterial infections of the face and neck--practical considerations. *Laryngoscope.* 1981;91:1714-26.
28. Tarantino L, Giorgio A, de Stefano G, Farella N, Perrotta A, Esposito F. Disseminated mycobacterial infection in AIDS patients: abdominal US features and value of fine-needle aspiration biopsy of lymph nodes and spleen. *Abdom Imaging.* 2003;28:602-8.
29. Lindeboom JA, Prins JM, Bruijnesteijn van Coppenraet ES, Lindeboom R, Kuijper EJ. Cervicofacial lymphadenitis in children caused by *Mycobacterium haemophilum*. *Clin Infect Dis.* 2005;41:1569-75.
30. Lindeboom JA. Diagnosis and treatment of nontuberculous mycobacterial cervicofacial lymphadenitis in children: a prospective multicenter, multidisciplinary study in the Netherlands. 2006;Thesis.
31. Robson CD, Hazra R, Barnes PD, Robertson RL, Jones D, Husson RN. Nontuberculous mycobacterial infection of the head and neck in immunocompetent children: CT and MR findings. *AJNR Am J Neuroradiol.* 1999;20:1829-35.
32. Pumberger W, Hallwirth U, Pawlowsky J, Pomberger G. Cervicofacial lymphadenitis due to atypical mycobacteria: a surgical disease. *Pediatr Dermatol.* 2004;21:24-9.
33. Grange JM, Yates MD, Pozniak A. Bacteriologically confirmed non-tuberculous mycobacterial lymphadenitis in south east England: a recent increase in the number of cases. *Arch Dis Child.* 1995;72:516-7.
34. Vu TT, Daniel SJ, Quach C. Nontuberculous mycobacteria in children: a changing pattern. *J Otolaryngol.* 2005;34(Suppl 1):S40-4.
35. Howell N, Heaton PA, Neutze J. The epidemiology of nontuberculous mycobacterial lymphadenitis affecting New Zealand children 1986-95. *N Z Med J.* 1997;110:171-3.
36. Haverkamp MH, Arend SM, Lindeboom JA, Hartwig NG, van Dissel JT. Nontuberculous mycobacterial infection in children: a 2-year prospective surveillance study in the Netherlands. *Clin Infect Dis.* 2004;39:450-6.
37. Saitz EW. Cervical lymphadenitis caused by atypical mycobacteria. *Pediatr Clin North Am.* 1981;28:823-39.
38. Wolinsky E. Mycobacterial lymphadenitis in children: a prospective study of 105 nontuberculous cases with long-term follow-up. *Clin Infect Dis.* 1995;20:954-63.
39. Tortoli E, Rindi L, Goh KS, Katila ML, Mariottini A, Mattei R, Mazzarelli G, Suomalainen S, Torkko P, Rastogi N. *Mycobacterium florentinum* sp. nov., isolated from humans. *Int J Syst Evol Microbiol.* 2005;55:1101-6.

40. De Baere T, Moerman M, Rigouts L, Dhooge C, Vermeersch H, Verschraegen G, Vaneechoutte M. *Mycobacterium interjectum* as causative agent of cervical lymphadenitis. J Clin Microbiol. 2001;39:725-7.
41. Wolinsky E. Mycobacterial diseases other than tuberculosis. Clin Infect Dis. 1992;15:1-10.
42. Jindal N, Devi B, Aggarwal A. Mycobacterial cervical lymphadenitis in childhood. Indian J Med Sci. 2003;57:12-5.
43. Primm TP, Lucero CA, Falkinham JO 3rd. Health impacts of environmental mycobacteria. Clin Microbiol Rev. 2004;17:98-106.
44. United States Office of Science and Technology EPA-822-B-01-007, Mycobacteria: Health Advisory Environmental Protection Office of Water August 1999 Agency Washington, DC 20460. website: <http://www.epa.gov>.
45. Meier JL, Beekmann SE. Mycobacterial and fungal infections of bone and joints. Curr Opin Rheumatol. 1995;7:329-36.
46. Olsen RJ, Cernoch PL, Land GA. Mycobacterial synovitis caused by slow-growing nonchromogenic species: eighteen cases and a review of the literature. Arch Pathol Lab Med. 2006;130:783-91.
47. Callaghan R, Allen M. *Mycobacterium malmoeense* infection of the knee. Ann Rheum Dis. 2003;62:1047-8.
48. Nakamura T, Yamamura Y, Tsuruta T, Tomoda K, Sakaguchi M, Tsukano M. *Mycobacterium kansasii* arthritis of the foot in a patient with systemic lupus erythematosus. Intern Med. 2001;40:1045-9.
49. Loddenkemper K, Enzweiler C, Loddenkemper C, Backhaus M, Burmester GR, Buttgereit F. Granulomatous synovialitis with erosions in the shoulder joint: a rare case of polyarthritis caused by *Mycobacterium kansasii*. Ann Rheum Dis. 2005;64:1088-90.
50. Graham DY, Markesich DC, Yoshimura HH. Mycobacteria and inflammatory bowel disease. Results of culture. Gastroenterology. 1987;92:436-42.
51. Tzen CY, Wu TY, Tzen CY. Detection of mycobacteria in Crohn's disease by a broad spectrum polymerase chain reaction. J Formos Med Assoc. 2006;105:290-8.
52. Bull TJ, McMinn EJ, Sidi-Boumedine K. Detection and verification of *Mycobacterium avium* ssp. *paratuberculosis* in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. J Clin Microbiol. 2003;41:2915-23.
53. Autschbach F, Eisold S, Hinz U. High Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* IS900 DNA in gut tissues from individuals with Crohn's disease. Gut. 2005;54: 944-49.
54. Ryan P, Bennett MW, Aarons S, Shanahan F. PCR detection of *Mycobacterium paratuberculosis* in Crohn's disease granulomas isolated by laser capture microdissection. Gut. 2002;51:665-70.
55. Chacon O, Bermudez LE, Barletta RG. Johne's Disease, inflammatory bowel disease, and *Mycobacterium paratuberculosis*. Annual Review of Microbiology 2004;58:329-363.
56. Feller M, Huwiler K, Stephan R, Altpeter E, Shang A, Furrer H, Pfyffer GE, Jemmi T, Baumgartner A, Egger M. *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease: a systematic review and meta-analysis. Lancet Infect Dis. 2007;7:607-13.
57. Chamberlin WM, Naser SA. Integrating theories of the etiology of Crohn's disease. On the etiology of Crohn's disease: questioning the hypotheses. Med Sci Monit. 2006;12:RA27-33.
58. Wong AK, Lam DS. *Mycobacterium chelonae* keratitis: report of a case and review of the literature. Yan Ke Xue Bao. 1998;14:156-63. Abstract.
59. Gusmao FA, Alvarenga L, Barbosa L, Sampaio J, Leao SC, Hofling-Lima AL, Freitas D. Deep stromal mycobacterial keratitis: viable bacteria after six months of treatment: case report and literature review. Arq Bras Oftalmol. 2005;68:551-3.
60. Ding LW, Lai CC, Lee LN, Hsueh PR. Disease caused by non-tuberculous mycobacteria in a university hospital in Taiwan, 1997-2003. Epidemiol Infect. 2006;1-8.
61. Malecha MA, Doughman DJ. *Mycobacterium chelonae* keratitis associated with soft contact lens wear. CLAO J. 2002;28:228-30. Abstract.
62. Rodriguez B, Holzinger KA, Le LH, Winkle RK, Allen RD. *Mycobacterium chelonae* keratitis after laser-assisted subepithelial keratectomy. J Cataract Refract Surg. 2006;32:1059-61.

63. John T, Velotta E. Nontuberculous (atypical) mycobacterial keratitis after LASIK: current status and clinical implications. *Cornea*. 2005;24:245-55.
64. Lee SB, Oliver KM, Strube YN, Mohan SK, Slomovic AR. Fourth-generation fluoroquinolones in the treatment of mycobacterial infectious keratitis after laser-assisted in situ keratomileusis surgery. *Can J Ophthalmol*. 2005;40:750-3.
65. Ford JG, Huang AJ, Pflugfelder SC, Alfonso EC, Forster RK, Miller D. Nontuberculous mycobacterial keratitis in south Florida. *Ophthalmology*. 1998;105:1652-8.
66. Douglas RS, Cook T, Shorr N. Lumps and bumps: late postsurgical inflammatory and infectious lesions. *Plast Reconstr Surg*. 2003;112:1923-8.
67. Garman ME, Orenge I. Unusual infectious complications of dermatologic procedures. *Dermatol Clin*. 2003;21:321-35.
68. Kalita JB, Rahman H, Baruah KC. Delayed post-operative wound infections due to non-tuberculous *Mycobacterium*. *Indian J Med Res*. 2005;122:535-9.
69. Takemoto N, Kohiyama R, Tsuboi J, Sasaki K, Sakurabayashi I, Miyata M. A case of a patient with post-operative empyema due to *Mycobacterium chelonae*. *Kyobu Geka*. 1996;49:301-5. Abstract.
70. Angenent LT, Kelley ST, St Amand A, Pace NR, Hernandez MT. Molecular identification of potential pathogens in water and air of a hospital therapy pool. *Proc Natl Acad Sci U S A*. 2005;102:4860-5.
71. Galassi L, Donato R, Tortoli E, Burrini D, Santianni D, Dei R. Nontuberculous mycobacteria in hospital water systems: application of HPLC for identification of environmental mycobacteria. *J Water Health*. 2003;1:133-9.
72. Kline S, Cameron S, Streifel A, Yakus MA, Kairis F, Peacock K, Besser J, Cooksey RC. An outbreak of bacteremias associated with *Mycobacterium mucogenicum* in a hospital water supply. *Infect Control Hosp Epidemiol*. 2004;25:1042-9.
73. Conger NG, O'Connell RJ, Laurel VL, Olivier KN, Graviss EA, Williams-Bouyer N, Zhang Y, Brown-Elliott BA, Wallace RJ Jr. *Mycobacterium simae* outbreak associated with a hospital water supply. *Infect Control Hosp Epidemiol*. 2004;25:1050-5.
74. Katoch VM. Infections due to non-tuberculous mycobacteria (NTM). *Indian J Med Res*. 2004;120:290-304.
75. Falkinham JO 3rd. Nontuberculous mycobacteria in the environment. *Clin Chest Med*. 2002;23:529-51.
76. Pai HH, Chen WC, Peng CF. Isolation of non-tuberculous mycobacteria from hospital cockroaches (*Periplaneta americana*). *J Hosp Infect*. 2003;53:224-8.
77. Johnson PDR, Azuolas J, Lavender CJ, Wishart E, Stinear TP, Hayman JA, et al. *Mycobacterium ulcerans* in mosquitoes captured during outbreak of Buruli ulcer, southeastern Australia. *Emerg Infect Dis*. 2007;13:1653-60.
78. Phillips MS, von Reyn CF. Nosocomial infections due to nontuberculous mycobacteria. *Clin Infect Dis*. 2001;33:1363-74.
79. Falkinham JO 3rd. Mycobacterial aerosols and respiratory disease. *Emerg Infect Dis*. 2003;9:763-7.
80. Ryll R, Kumazawa Y, Yano I. Immunological properties of trehalose dimycolate (cord factor) and other mycolic acid-containing glycolipids--a review. *Microbiol Immunol*. 2001;45:801-11.
81. Fujita Y, Naka T, Doi T, Yano I. Direct molecular mass determination of trehalose monomycolate from 11 species of mycobacteria by MALDI-TOF mass spectrometry. *Microbiology*. 2005;151:1443-52.
82. Chen JM, German GJ, Alexander DC, Ren H, Tan T, Liu J. Roles of *Lsr2* in Colony Morphology and Biofilm Formation of *Mycobacterium smegmatis*. *J Bacteriol*. 2006;188:633-641.
83. Nguyen L, Pieters J. The Trojan horse: survival tactics of pathogenic mycobacteria in macrophages. *Trends Cell Biol*. 2005;15:269-76.
84. Briken V, Porcelli SA, Besra GS, Kremer L. Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response. *Mol Microbiol*. 2004;53:391-403.
85. Haverkamp MH, van Dissel JT, Holland SM. Human host genetic factors in nontuberculous mycobacterial infection: lessons from single gene disorders affecting innate and adaptive immunity and lessons from molecular defects in interferon-gamma-dependent signaling. *Microbes Infect*. 2006;8:1157-66.

86. Tonjum T, Welty DB, Jantzen E, Small PL. Differentiation of *Mycobacterium ulcerans*, *M. marinum*, and *M. haemophilum*: mapping of their relationships to *M. tuberculosis* by fatty acid profile analysis, DNA-DNA hybridization, and 16S rRNA gene sequence analysis. *J Clin Microbiol*. 1998;36:918-25.
87. Dobos KM, Quinn FD, Ashford DA, Horsburgh CR, King CH. Emergence of a unique group of necrotizing mycobacterial diseases. *Emerg Infect Dis*. 1999;5:367-78.
88. Kent ML, Whipps CM, Matthews JL, Florio D, Watral V, Bishop-Stewart JK, Poort M, Bermudez L. Mycobacteriosis in zebrafish (*Danio rerio*) research facilities. *Comp Biochem Physiol C Toxicol Pharmacol*. 2004;138:383-90.
89. Collins HL. The role of iron in infections with intracellular bacteria. *Immunol Lett*. 2003;85:193-5.
90. Schaible UE, Kaufmann SH. Iron and microbial infection. *Nat Rev Microbiol*. 2004;2:946-53. Review.
91. Daniel AK, Lee RE, Portaels F, Small PL. Analysis of *Mycobacterium* species for the presence of a macrolide toxin, mycolactone. *Infect Immun*. 2004;72:123-32.
92. Ranger BS, Mahrous EA, Mosi L, Adusumilli S, Lee RE, Colorni A, Rhodes M, Small PL. Globally distributed mycobacterial fish pathogens produce a novel plasmid-encoded toxic macrolide, mycolactone F. *Infect Immun*. 2006;74:6037-45.
93. Yip MJ, Porter JL, Fyfe JA, Lavender CJ, Portaels F, Rhodes M, Kator H, Colorni A, Jenkin GA, Stinear T. Evolution of *Mycobacterium ulcerans* and other mycolactone-producing mycobacteria from a common *Mycobacterium marinum* progenitor. *J Bacteriol*. 2007;189:2021-9.
94. Hwang JH, Koh WJ, Kim EJ, Kang EH, Suh GY, Chung MP, Kim H, Kwon OJ. Partial interferon-gamma receptor deficiency and non-tuberculous mycobacterial lung disease. *Tuberculosis*. 2007;87:166-71.
95. Ottenhoff TH, Verreck FA, Lichtenauer-Kaligis EG, Hoeve MA, Sanal O, van Dissel JT. Genetics, cytokines and human infectious disease: lessons from weakly pathogenic mycobacteria and salmonellae. *Nat Genet*. 2002;32:97-105.
96. van de Vosse E, Lichtenauer-Kaligis EG, van Dissel JT, Ottenhoff TH. Genetic variations in the interleukin-12/interleukin-23 receptor (beta1) chain, and implications for IL-12 and IL-23 receptor structure and function. *Immunogenetics*. 2003;54:817-29.
97. van de Vosse E, de Paus RA, van Dissel JT, Ottenhoff TH. Molecular complementation of IL-12Rbeta1 deficiency reveals functional differences between IL-12Rbeta1 alleles including partial IL-12Rbeta1 deficiency. *Hum Mol Genet*. 2005;14:3847-55.
98. Subcommittee of the Joint Tuberculosis Committee of the British Thoracic Society* Management of opportunist mycobacterial infections: Joint Tuberculosis Committee guidelines 1999. *Thorax*. 2000;55:210-218.
99. Doucet-Populaire F, Buriankova K, Weiser J, Pernodet JL. Natural and acquired macrolide resistance in mycobacteria. *Curr Drug Targets Infect Disord*. 2002;2:355-70.
100. Johnson R, Streicher EM, Louw GE, Warren RM, van Helden PD, Victor TC. Drug resistance in *Mycobacterium tuberculosis*. *Curr Issues Mol Biol*. 2006;8:97-111.
101. Forget EJ, Menzies D. Adverse reactions to first-line antituberculosis drugs. *Expert Opin Drug Saf*. 2006;5:231-49.
102. Griffith DE. Nontuberculous mycobacteria. *Curr Opin Pulm Med*. 1997;3:139-45. Review. Abstract.
103. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. *Am J Respir Crit Care Med*. 2003;167:1472-7.
104. Mussaffi H, Rivlin J, Shalit I, Ephros M, Blau H. Nontuberculous mycobacteria in cystic fibrosis associated with allergic bronchopulmonary aspergillosis and steroid therapy. *Eur Respir J*. 2005;25:324-8.
105. Luong A, McClay JE, Jafri HS, Brown O. Antibiotic therapy for nontuberculous mycobacterial cervicofacial lymphadenitis. *Laryngoscope*. 2005;115:1746-51.
106. Lindeboom JA, Kuijper EJ, Bruijnesteijn van Coppenraet ES, Lindeboom R, Prins JM. Surgical excision versus antibiotic treatment for nontuberculous mycobacterial cervicofacial lymphadenitis in children: a multicenter, randomized, controlled trial. *Clin Infect Dis*. 2007;44:1057-64.
107. Panesar J, Higgins K, Daya H, Forte V, Allen U. Nontuberculous mycobacterial cervical adenitis: a ten-year retrospective review. *Laryngoscope*. 2003;113:149-54.

108. Polesky A, Grove W, Bhatia G. Peripheral tuberculous lymphadenitis: epidemiology, diagnosis, treatment, and outcome. *Medicine (Baltimore)*. 2005;84:350-62.

