Recommendations of the German Central Committee against Tuberculosis (DZK) and the German Respiratory Society (DGP) for the Diagnosis and Treatment of Non-tuberculous Mycobacterioses

Empfehlungen zur Diagnostik und Therapie nichttuberkulöser Mykobakteriosen des Deutschen Zentralkomitees zur Bekämpfung der Tuberkulose (DZK) und der Deutschen Gesellschaft für Pneumologie und Beatmungsmedizin (DGP)

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Abstract

Non-tuberculous mycobacterioses comprise a group of diseases caused by mycobacteria which do not belong to the *Mycobacterium (M.) tuberculosis-complex* and are not ascribed to *M. leprae*. These mycobacteria are characterized by a broad variety as to environmental distribution and adaptation. Some of the species may cause specific diseases, especially in patients with underlying immunosuppressive diseases, chronic pulmonary diseases or genetic predisposition, respectively. Worldwide, a rising prevalence and significance of non-tuberculous mycobacterioses is recognized. The present recommendations summarise current aspects of epidemiology, pathogenesis, clinical aspects, diagnostics – especially microbiological methods including susceptibility testing –, and specific treatment for the most relevant species. Diagnosis and treatment of non-tuberculous mycobacterioses during childhood and in HIV-infected individuals are described in separate chapters.

Zusammenfassung

Die nichttuberkulösen Mykobakteriosen umfassen eine Gruppe von Erkrankungen, die von Mykobakterien verursacht werden, die nicht dem *Mycobacterium (M.) tuberculosis*-Komplex und nicht *M. leprae* zugerechnet werden und durch eine breite Vielfalt in Hinsicht auf ihr Vorkommen und ihre Anpassungen an spezifische Umweltbedingungen charakterisiert sind. Einige Spezies können definierte Krankheitsbilder insbesondere bei Patienten mit systemischer Immunsuppression, vorbestehenden Lungenerkrankungen oder genetisch bedingter erhöhter Empfänglichkeit hervorrufen. Weltweit wird eine Zunahme der Prävalenz und der Bedeutung dieser Erregergruppe beobachtet. Die vorliegenden Empfehlungen fassen aktuelle Aspekte der Epidemiologie, der Pathogenese, Klinik, Diagnostik einschließlich mikrobiologischer Diagnostik und Resistenztestung sowie der speziesabhängigen Therapie bei nicht-tuberkulösen Mykobakteriosen zusammen. Außerdem werden die besonderen Aspekte der Diagnostik und Therapie im Kindesalter und bei HIV-infizierten Patienten dargestellt.

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1 Introduction

Worldwide, in particular in countries with low tuberculosis prevalence, an increase in prevalence and importance of non-tuberculous mycobacteria (NTM) is observed [1 – 3]. NTM have been known almost as long as *M. tuberculosis*, but its clinical relevance has only been realized since the 1950s. In particular HIV infection, which before the introduction of antiretroviral therapy had been complicated often by *M. avium-complex* (MAC) disease, focussed the interest on NTM [4].

In contrast to the decline of tuberculosis, NTM play an increasing role in Germany during several years, mainly in non-HIV positive patients [2, 5 – 7].

Many aspects of the epidemiology, pathogenesis, diagnosis and therapy are still not clarified. These recommendations presented are based mainly on the comprehensive statement of the American Thoracic Society (ATS) which was developed in 2007 together with the Infectious Diseases Society of America (IDSA) [1]. The joint recommendations of the German Central Committee against Tuberculosis (DZK) and the German Respiratory Society (DGP) include in addition the extensive more recent literature and depict single items of the American Statement in a differentiated form.

The term NTM refers to a large group of environmental mycobacteria, characterized by a wide diversity in terms of their occurrence and their adaptation to specific environmental conditions. NTM are mycobacteria which do not belong the *Mycobacterium (M.) tuberculosis-complex* and *M. Leprae*. This is also mirrored in the development of the nomenclature: besides environmental mycobacteria, other synonyma have been used as ubiquitous, opportunistic, “Mycobacteria other than tuberculosis” (MOTT) and atypical mycobacteria. The present recommendations use predominantly the term NTM.

NTM are widely spread; they colonise many different environmental sites, such as soil, surface and drinking water [8 – 10]. Via water they enter domestic water pipes where they can occur in biofilms with high bacterial counts [8 – 11]. Furthermore, NTM can be detected, amongst others in food such as pasteurized milk and cheese, but not homogenized milk [12 – 15]. As a consequence there is a constant human contact with these ubiquitous mycobacteria [9].

To date, more than 150 species of NTM have been validly described (www.bacterio.cict.fr/m/mycobacterium.html as of 02/2015). The most frequently isolated species from patient specimens are listed in **Table 1**.

Non-tuberculous mycobacteriosis must be distinguished from NTM (especially *M. avium-complex*) -induced extrinsic allergic alveolitis (e.g. “hot tub lung”) [17 – 19]. In addition, *M. immunogenum* in metalworking fluids is a trigger for extrinsic allergic alveolitis in metal workers in the automobile industry [20].

2 Epidemiology and importance of non-tuberculous mycobacteria (NTM)

During the last three decades the prevalence of NTM lung disease has increased in some regions of the world, in part dramatically [3]. Reasons for this are assumed to be an aging population with increased comorbidities, HIV infection, frequent use of immunosuppressive drugs, more sensitive laboratory diagnostics, and possibly a lower cross-reactive immunity to mycobacteria as a result of the decrease in tuberculosis infections [1 – 3].

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Table 1 Common slowly and rapidly growing non-tuberculous mycobacteria, often isolated from clinical specimens (modified from [16] (see Chapter 8, Appendix 1: "Microbiological and therapeutic features of selected species of mycobacteria," p. 264).

Slowly growing mycobacteria	Rapidly growing mycobacteria
M. arupense	*M. abscessus ssp. abscessus*
M. asiaticum	*M. abscessus ssp. bolettii*
M. avium	*M. alvei*
M. bohemicum	*M. aurum*
M. branderi	*M. boenickei*
M. celatum	*M. brumae*
M. chimaera	*M. chelonae*
M. europaeum	*M. confluentis*
M. florentinum	*M. elephantis*
M. genavense	*M. fortuitum*
M. gordonae	*M. goodii*
M. haemophilum	*M. holsaticum*
M. heckeshornense	*M. immunogenum*
M. interjectum	*M. iranicum sp. nov.*
M. intermedium	*M. margeritense*
M. intracellulare	*M. mucogenicum*
M. kansasii	*M. peregrinum*
M. kubicae	*M. phocaicum*
M. lentiflavum	*M. septicum*
M. malmoense	*M. thermoresistible*
M. marinum	
M. nebraskense	
M. palustre	
M. saskatchewanse	
M. scrofulaceum	
M. shimodei	
M. simiae	
M. szulgai	
M. ulcerans	
M. vulnerans	
M. xenopi	

In Canada the reported prevalence of NTM between 1997 and 2003 increased from 9.1/100,000 to 14.1/100,000 population [21]. In the US, between 2004 – 2006, there was a combined incidence of 5.5/100,000 person-years observation (PYO) (for those aged over 60, the incidence was 26.7/100,000 PYO) [22], and in another study, a 8.6/100,000 population 2-year period prevalence was observed [23]. Winthrop et al. in their study, which was based on a large cohort found that the microbiological criteria of ATS/IDSA alone in comparison with the full diagnostic criteria indicated in a high percentage (86 %) a true NTM disease [23]. In a further US study of individuals over 65 years old there was an increase in prevalence from 20 to 47/100,000 between 1997 and 2007 [9]. In Australia, between 1999 and 2005, the prevalence of pulmonary disease rose from 2.2 to 3.3 per 100,000 population [24]. In a university hospital in Taiwan, between 2000 and 2008 an increase in the prevalence of pulmonary NTM disease from 1.3 to 7.9 per 100,000 patients was observed [25].

In Europe generally lower numbers are reported [2, 3]. In England, Wales and Northern Ireland between 1995 and 2006, the number of NTM diseases rose from 0.9 to 2.9 per 100,000 population [26]. In the Netherlands, the incidence of pulmonary NTM disease has also increased and was conservatively estimated in 2008 at 1.7 per 100,000 [27, 28] and in Denmark the incidence of NTM disease was estimated at 1.08 per 100,000 [29]. From Croatia, an increase of NTM isolates from 235 cases in 2006 to 416 in 2010 has been reported [30], which, due to the applied microbiological criteria, can be considered in most cases as true diseases [23, 31]. The most clinically relevant NTM species present there were *M. xenopi* and *M. avium*. The observation that in Croatia the incidence of probable pulmonary disease was twice as high in coastal urban areas than in the countryside was possibly explained by differences in local water supply [30].

For Germany there are no estimates of prevalence in adults (epidemiological data for children are listed in Chapter 7, "Diagnosis and therapy of non-tuberculous mycobacteriosis in children," p. 262). Because NTM is not transmissible from person to person there is, under the Infection Protection Act (IfSG), no obligation to report to the health authorities, and there is no systematic surveillance (contact tracing and special infection control measures for non-tuberculous mycobacteriosis are generally not necessary). Given this situation, and the multiple non-specific clinical manifestations (see Chapter 4, "Diagnosis," p. 255), it is almost impossible to achieve a clear picture of the epidemiology of diseases caused by NTM [2].

The evaluation of laboratory data (for example, data from the National Reference Center for Mycobacteria, Borstel) does not allow clinically relevant conclusions, as the sole detection of NTM is not necessarily equivalent with disease. Thus, the most frequently isolated species (such as *M. gordonae* and *M. avium-complex*) are widespread in the environment. In addition, laboratory data are influenced by many other factors, such as the availability of basic laboratory methods for identification of the species, but also, by new descriptions and changes in nomenclature (see Chapter 4.3, "Microbiological diagnostics and drug susceptibility testing," p. 256).

The assessment of epidemiological trends is further complicated by changes that significantly affect the structure of the examined population over time. These include demographic factors like increased life expectancy and gender distribution, changing exposures (such as the popularity of hot tubs), and variations in prevalence of disease-predisposing factors (in particular, severe immunosuppression, such as HIV infection, or immunosuppressive therapy, and chronic lung diseases in old age), but, too, the increasing availability and higher quality of diagnostic methods, and, – not least – the attention to diseases caused by NTM plays a role [32 – 36]. Furthermore, regional differences must be taken into account, because both prevalence and the prevailing NTM species may vary from region to region [1, 3, 10, 37]. As an example, Hoefsloot et al. found in their global snapshot on the geographic distribution of NTM that isolation of *M. xenopi* from respiratory materials is limited mainly to certain regions (Belgium, France, Croatia, Hungary) [38].

A direct comparison of clinical trials is often fails due to the lack of application of uniform diagnostic criteria, unprecise pathogen characterization, lack of clinical reference data, varying inclusion criteria and study populations (outpatient, inpatient, risk groups), and (at least in earlier studies) the unknown proportion of HIV-positive patients [9, 39 – 41]. Disregarding the difficulties in assessing the correct epidemiological situation, NTM disease represents a relevant problem because its diagnosis is often complex, requires lengthy, costly and care-intensive treatment and often is associated with undesirable adverse drug effects [42].

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3 Pathogenesis

The transmission of NTM is predominantely due to inhalation of aerosols or dust particles contaminated with NTM [1]. It can also be a result of contaminated water or soil, as suggested in the case of cervical lymphadenitis in young children, or wound infections. Transmission of *M. xenopi* and *M. avium* is also possible with contaminated dust from a clarifier, and fertilization with sewage sludge [43]. Transmission of NTM from person to person has not been detected so far. However, results of whole genome sequence analysis of *M. abscessus* isolates from patients with cystic fibrosis (CF) showed the possibility of transmission of certain strains [44]. Since most NTM occur in the environment, the detection of NTM from non-sterile human specimens (e.g. sputum) may also be caused by a contamination and does not necessarily imply disease [1,45,46] (definition of contamination, colonization or infection, see **Table 2**).

Within the spectrum of mycobacterial species, differences in pathogenicity of different NTM species are known [1,16,45]. Species as *M. gordonae* or *M. fortuitum*, which often are found in the environment, have a very low pathogenicity, whereas species such as *M. ulcerans* and *M. marinum* are in most cases associated with disease. Some NTM species can cause defined disease patterns (non-tuberculous mycobacteriosis) in non-HIV-infected patients (see **Table 3**) as well as in the course of HIV infection which will be addressed in a separate chapter (Chapter 6, "Non-tuberculous mycobacteriosis in HIV infection," p. 260).

3.1 Predisposing factors

Certain local or general risk factors, and underlying diseases, are important prerequisites for the development of NTM disease. The main predisposing factors are listed in **Table 4**.

Patients with neither local nor systemic risk factors have also been found [49,50]. However, a recently published study provided evidence that cases of pure pulmonary manifestations may be a result of defective mucociliary clearance [51] (see below). In addition, the different virulence of individual species seems to play a role in disease development. For example, the detection of *M. kansasii* is often associated with the clinical criteria for disease [45,52]. With *M. avium*, however, it is advisable to verify in each single case the presence of a disease requiring treatment (**Fig. 1**) [45].

3.1.1 Local risk factors

In contrast to tuberculosis, the pathogenesis of bronchopulmonary disease due to NTM requires local tissue abnormalities or changes in the majority of HIV-negative patients [48]. Most local bronchopulmonary risk factors for non-tuberculous mycobacteriosis (see **Table 4**) are COPD, bronchiectasis, cystic fibrosis (CF), previous tuberculosis with residuals, silicosis and other pneumoconiosis, and alveolar proteinosis [53]. In difficult-to-treat asthma an additional infection with NTM should be considered [54]. Moreover physical characteristics such as a tall, slim physique or thoracic deformities such as scoliosis, pectus excavatum or ankylosing spondylitis, seem to predispose to non-tuberculous mycobacteriosis, possibly due to an impairment of the bronchial clearance function [45,51,55,56]. Diseases are more frequently observed under regular use of inhaled corticosteroids. The risk varies with dose and the type of the steroide (higher with fluticasone compared to budesonide) [57].

Table 2 Definitions of terms relating to the presence of NTM in respiratory materials [1,45,46].

contamination	(environmental) soiling of the specimen from the outside by NTM
colonization	presence of NTM in a specimen from the respiratory tract without formation of granuloma
infection without signs of disease	formation of specific granulation tissue, but without symptoms
infection with signs of disease	formation of specific granulation tissue with symptoms

Table 3 Diseases caused by NTM, and frequently isolated species in non-HIV-infected adults (according to [1]).

Disease	Main pathogens
pulmonary infection	*M. avium-complex, M. kansasii, M. xenopi, M. malmoense*
skin and soft tissue infections	*M. marinum, M. ulcerans, M. chelonae, M. abscessus*
eye infections	*M. chelonae, M. abscessus, M. fortuitum*
skeletal infections (osteomyelitis)	*M. abscessus*
otitis media (middle ear infection), mastoiditis	*M. abscessus, M. kansasii, M. xenopi*
cervical lymphadenitis (mainly in children)	*M. avium-complex, M. malmoense, M. scrofulaceum, M. haemophilum*
catheter- or device-associated infections	*M. abscessus, M. chelonae*
cystic fibrosis (pulmonary infections)	*M. abscessus, M. avium-complex, M. chelonae*
disseminated disease (with immunosuppression or genetic predisposition)	*M. avium-complex*
post transplantation	*M. avium-complex, M. kansasii*

Table 4 Predisposing factors for non-tuberculous mycobacteriosis [1,47,48].

pre-existing lung disease	COPD, asthma, bronchiectasis, lung cancer, smoking, recurrent aspiration with gastro-esophageal reflux disease (GERD), inhaled corticosteroids, pulmonary fibrosis, cystic fibrosis, thoracic deformities such as ankylosing spondylitis, scoliosis, previous tuberculosis, postinfectious residual cavitation, bulla or cyst, pneumoconiosis (silicosis), alveolar proteinosis
systemic immune deficiency	HIV infection (in particular peripheral CD4+ cell count < 50/µl), status after organ transplantation, malignancies (hematological cancer), alcoholism, cachexia, immunosuppressive therapy (e.g., TNF-alpha inhibitors, corticosteroids), teething (in children with cervical lymphadenitis), age (young children and the elderly), male gender*, diabetes mellitus*, renal failure*
increased genetic susceptibility due to mutations of the following genes	interferon gamma receptor gene, interleukin-12 receptor gene, Signal Transducer and Activator of Transcription 1 (STAT 1) transmembrane conductance regulator gene (cystic fibrosis), alpha 1-antitrypsin gene

* suspected or possible risk factor

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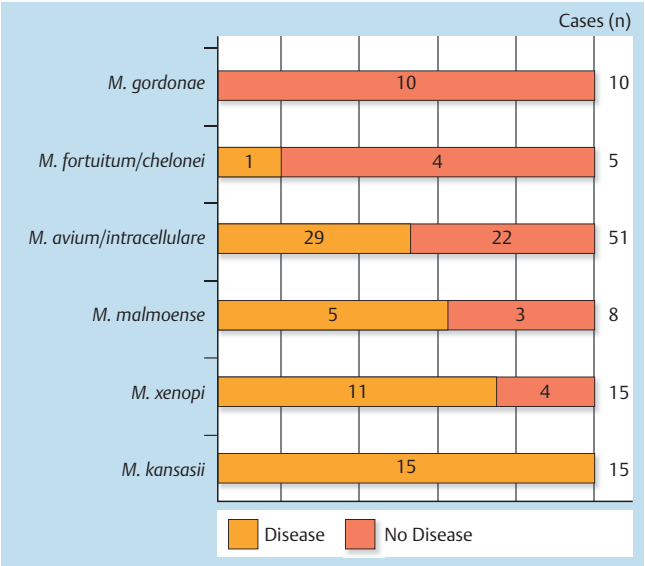


Fig. 1 Morbidity (in pulmonary infections) with the major non-tuberculous mycobacterial species (n = 104) [45].

The assumption that women often consciously avoid expectoration of sputum ("Ladies do not spit") has prompted Reich and Johnson to denote the following constellation according to Oscar Wilde's Victorian figure of Lady Windermere: That is, a disease of older women of tall and slim physique, without pre-existing pulmonary conditions, with initial involvement of the lingula or middle lobe by *M. avium-complex* [58,59].

Gastro-esophageal reflux disease (GERD) is frequently detected in patients with pulmonary infections caused by NTM and correlates with the extent of bronchiectasis. The detection of acid-fast bacilli (NTM) in sputum is more frequent in patients with GERD, too [60].

In patients with predisposing pulmonary factors ("terrain factor" according to Forschbach [61]) a ventilation disorder with worsening of bronchial clearance function due to the retention of secretions seems to be crucial. Here the risk factors for chronic bronchopulmonary infections/colonizations do not differ from that of other bacteria (e.g. *Pseudomonas aeruginosa*, etc.). Taking into account possible laboratory contamination, most patients with local bronchopulmonary changes in whom NTM are detected, should be considered not only to be colonized but probably to be infected [1,23]. Patients with cystic fibrosis (CF) have an increased risk for local NTM infections, especially through *M. abscessus* or *M. avium-complex* (MAC) [62–64]. It has been reported that in patients with cystic fibrosis (CF), despite repeated mycobacterial detection in sputum, no granulomas were found after autopsy [65]. Such findings suggest that an exclusive colonization without host reaction is possible.

Locally multiplied NTM may lead to the development of a tuberculosis-like disease picture with pulmonary infiltrates, possibly resulting in multiple cavities, too. Bronchogenic spread may lead to involvement of additional regions of the lung. However, clinically and radiologically speaking, the disease is usually less pronounced than in tuberculosis (see section 4.2, "Radiological manifestation," p. 256). The association between bronchiectasis and infection with *M. avium* is pathogenetically not definitely clarified. It is conceivable that the microbes prefer to colonize in pre-existing bronchiectatic areas, and likewise, that the bronchiectatic changes only are caused by a specific inflammation of the bronchial walls, similar to tuberculosis [66].

In addition, there are forms of the disease that resemble primary and post-primary manifestations of tuberculosis, in the absence of mitigating local defence factors.

Here, the intensity of exposure to NTM, the virulence of the pathogen, as well as age and genetic factors of the host may play a role. Besides pulmonary involvement the most frequent local manifestation, other local infections may occur (see ▶ **Table 3**). So, for example, skin, soft tissue, eye and skeletal infections can be caused by NTM [33,67], as well as cervical lymphadenopathy, which usually afflicts children under the age of five (see Chapter 7, "Diagnosis and therapy of non-tuberculous mycobacteriosis in children," p. 262).

Furthermore, infections due to various medical or surgical procedures (contaminated instruments, syringes, during operations, etc.) [1,68–70], or on other occasions such as foot baths at pedicure or tattooing [71,72] have been documented.

A non-tuberculous mycobacteriosis with a distinctly circumscribed disease presentation is the so-called "swimming pool granuloma," due to infection by *M. marinum*. Fishes are the main hosts, and thus the vectors for the transmission to humans. The source of *M. marinum* infections is contaminated water, usually in aquaria with diseased fish. Superficial skin wounds are the probable sufficient as ports of entry [73]. In some tropical regions of mainly West Africa the prevalence of Buruli ulcers with underlying infections with *M. ulcerans* suggests water as the major source. In addition, recent studies suggest a possible connection with aquatic insects [74].

3.1.2 Systemic risk factors

Deficiencies in systemic immunity may favour diseases caused by NTM (see ▶ **Table 4**). Thus, patients with HIV infection and CD4+ lymphocyte counts <50/µl are particularly vulnerable to a disseminated infection by NTM [1]. However, since the introduction of antiretroviral therapy, the number of HIV patients with mycobacteriosis has significantly decreased [75,76]. Those HIV-associated non-tuberculous mycobacteriosis that are still playing a substantial role are described separately in Chapter 6, "Non-tuberculous mycobacteriosis in HIV infection," p. 260.

Epidemiological studies show that, unlike in tuberculosis, diabetes mellitus is apparently not a greatly increased risk factor for the development of a non-tuberculous mycobacteriosis. However, local infections with NTM have been described at injection sites in insulin-dependent diabetics [69]. Chronic renal failure is considered a possible risk factor, according to retrospective analyses [77]. In the course of chronic renal failure, NTM disease can be due to invasive procedures such as peritoneal or hemodialysis [70,78]. Furthermore non-tuberculous mycobacteriosis are observed frequently in transplant patients [79].

Non-tuberculous mycobacterioses also occur in patients with chronic inflammatory diseases undergoing treatment with TNF-α inhibitors and other biologicals [80,81] – however –, about ten times less often compared to tuberculosis [82,83].

Diseases caused by NTM are also observed in association with systemic and inhaled glucocorticosteroid use [57,84].

The current state of knowledge on immune defect syndromes, such as IL12R deficiency, but also on other promoting systemic factors and assumed causes is summarized in recent review papers [47,48,85].

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Despite these clinical observations, the pathophysiological mechanisms are only partially understood. The predisposing underlying diseases such as COPD and bronchiectasis are to some extent widespread. However, since only a relatively small number of patients out of such large disease groups develop NTM disease, additional genetic host and pathogen components have to be suspected. A hereditary cause of lung diseases by NTM has been described, too [86,87].

It can be expected that the relationship with other predisposing factors will be revealed in the future [50,88].

Thus, Fowler et al. were able to find in their study on patients with non-tuberculous mycobacteriosis in whom CF or primary ciliary dyskinesia were excluded, that the mucociliary clearance was impaired in comparison with a control group of healthy subjects [51]. This could explain that patients with pulmonary manifestations, but without underlying immunodeficiency do not, or rarely, develop simultaneous manifestations of their non-tuberculous mycobacteriosis in other organs. This may also point to an additional hereditary disposition. The authors consider that the decreased NO production may offer a therapeutic approach by increasing the "NO-cyclic guanosine monophosphate (cGMP) pathway."

Genetic variability is increasingly detected within many species, but the significance of this in terms of pathogenicity is largely unknown [89]. Several genetic variants for *M. kansasii* have been described of which subspecies I has a worldwide distribution. Subspecies II seems to be detected increasingly in HIV patients, whereas the other, less common subspecies presumably cause disease more rarely [90].

In addition to the NTM that are common environmental contaminants, species such as *M. ulcerans*, *M. haemophilum* or *M. genavense* are probably much more host-specific. With *M. genavense*, infections of birds have been described, but there is no evidence of spread outside this specific host. *M. haemophilum* also has to date an unclear natural habitat and mode of transmission, but water reservoirs are considered a likely source of infection [91].

4 Diagnosis

Diagnosis of a disease caused by NTM is complex. The diagnostic criteria for a non-tuberculous mycobacterial infection are summarized in the 2007 statement of the ATS and the IDSA guidelines [1].

The following criteria are required for diagnosis of NTM disease:

- a compatible clinical and radiographic picture,
- the exclusion of other plausible causes of the clinical presentation,
- worsening symptoms despite treatment of an alternate diagnosis,
- the exclusion of contamination,
- detection of the same pathogen from non-sterile samples on multiple occasions,
- and in the case of pulmonary disease, if the specified clinical, radiological and microbiological criteria are met (see **Table 5**).

NTM in non-HIV-infected patients are usually found in sputum, but may also be recovered from other samples (see "Clinical specimens" in section 4.3, "Microbiological diagnostics and drug susceptibility testing," p. 256). Occasionally it may be necessary to obtain a specimen for histological diagnosis if the differential diagnosis is still unclear due to microbiological investigation without result. Bronchoscopy with bronchial aspiration with or without prior washing, peripheral catheter or needle biopsy, bronchoalveolar lavage and/or transbronchial forceps biopsy is the method of choice for obtaining specimens for histological evaluation. If necessary, CT-guided pulmonary biopsies can be performed if lung function allows this procedure (risk of pneumothorax with the development of an acute respiratory insufficiency).

Table 5 Clinical, radiological and microbiological criteria for the diagnosis of pulmonary disease caused by non-tuberculous mycobacteria (modified from [1]).

a) Clinical/radiological criteria (both must be met)
1. Bronchopulmonary symptoms, nodular or cavitary changes on chest X-ray, or multilocular bronchiectasis with multiple small nodular foci in high-resolution computed tomography (HRCT)
plus
2. Exclusion of other causes
b) Microbiological
1. Positive cultures from at least two separate expectorated sputum samples (If the results do not lead to a definite diagnosis, repeat sputum smears and cultures are recommended).
or
2. Positive culture from at least one sample taken from bronchoscopy (using bronchial washing/lavage)
or
3. Transbronchial or other lung biopsy with histopathologic findings typical of mycobacteria (ie. granulomatous inflammation or acid-fast bacilli) plus positive culture, or biopsy with evidence of mycobacterial histopathologic findings (ie. granulomatous inflammation or acid-fast bacilli) plus a positive culture from at least one or more other materials (sputum, bronchial).
4. Expert opinion should be sought when a rare mycobacterial species or common environmental contaminant is discovered.
5. Persons in whom a NTM infection is suspected, but do not satisfy the diagnostic criteria, should be monitored until a definitive diagnosis is made.
6. The diagnosis of NTM is not necessarily an indication for treatment. This must be decided after assessing the potential benefits and possible risks of treatment for each patient.

Histological examination shows the typical appearance of an epitheloid cell granulomatosis with or without necrosis (see **Fig. 2c**), which is indistinguishable from tuberculosis [92]. In case tissue specimens were obtained and not fixed in formalin, specific molecular tests may be performed in addition to microbiological culture examinations if NTM disease is suspected.

It should be noted that the tuberculin skin test cross-reacts with numerous mycobacterial species (false-positive), whereas the interferon-γ release assay (IGRA) is almost always (true-) negative. However, false-positive IGRA responses can occur with *M. flavescens*, *M. kansasii*, *M. marinum*, and *M. szulgai* infections [93,94].

The diagnosis of non-tuberculous mycobacterial infection requires accurate identification of the specific NTM species [95]. The increase of validly proven NTM species is due to sophisticated molecular-biological techniques [96]. As mentioned in the introduction, more than 150 different mycobacterial species have been documented (www.bacterio.cict.fr/m/mycobacterium.html, as of 02/2015) including a number of potential pathogens (see **Table 1**). Modern molecular diagnostics techniques enable an accurate species identification also with routinely performed methods (see chapter 4.3, "Microbiological diagnostics and drug susceptibility testing," p. 256).

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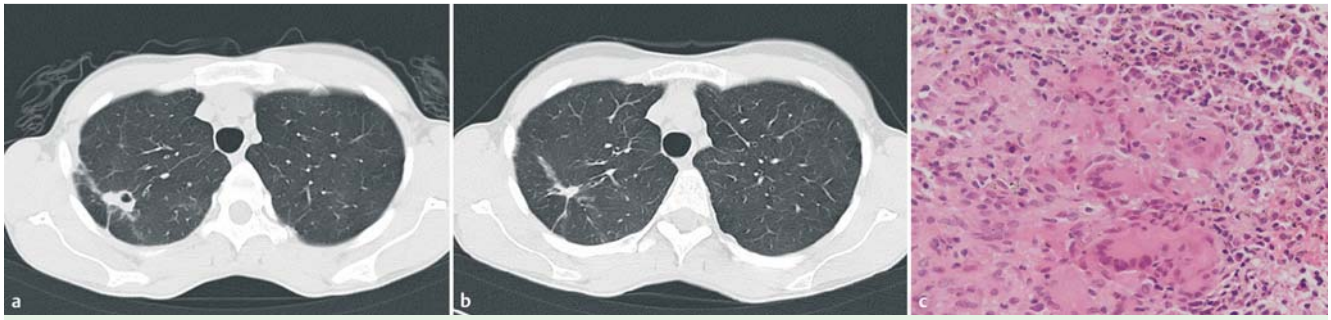


Fig. 2 **a – c** Non-tuberculous mycobacterial Infection by *M. avium* in the right upper lobe, resembling in appearance post-primary pulmonary tuberculosis, female, 29 years (CT). [Courtesy of Dr. Roland C. Bittner, Institute for Diagnostic and Interventional Radiology **a, b** and Priv.-Doz. Dr. Thomas Mairinger and Sergei Griff, Institute for the tissue diagnostics **c**, Helios Klinikum Emil von Behring]. **a** The patient had initially been treated for several months without bacteriological proof for suspected tuberculosis, but showed no regression. **c** CT-guided transthoracic needle biopsy revealed mass acid fast bacilli (molecular biology *M. avium*) and histologically epithelioid granulomas without necrosis. **b** Mycobacteriosis by *M. avium* was later confirmed on culture. The patient showed good response after 18 months of therapy, with a residual scar left.

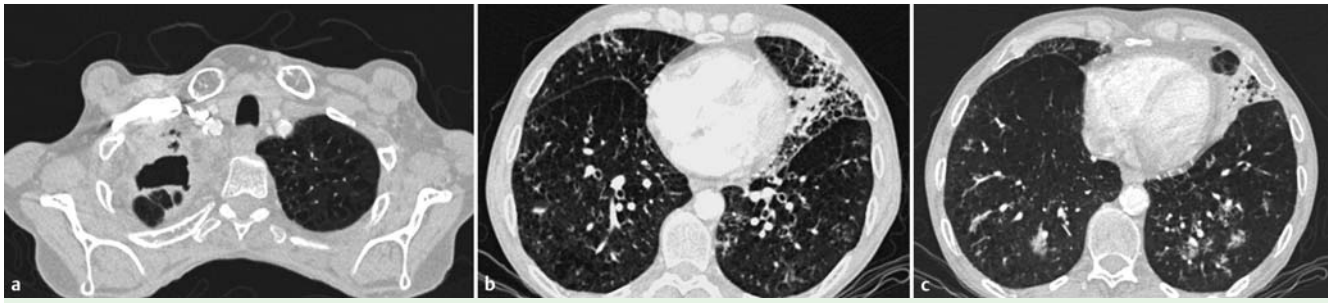


Fig. 3 **a – c** Non-tuberculous mycobacteriosis in a 59-year-old patient with severe pre-existing lung disease and *proof of mass M. malmoense*. **a** Cavities in the right upper lobe inside post-tuberculous residuals; **b** bronchiectasis in the lingula with surrounding infiltrate; **c** scattered lesions mainly in both lower lobes. [Courtesy of Dr. Roland C. Bittner, Institute for Diagnostic and Interventional Radiology, Helios Klinikum Emil von Behring].

The simultaneous detection of *M. tuberculosis* and NTM is difficult to interpret [97,98]. Here it needs to be evaluated carefully, whether in addition to tuberculosis another disease caused by NTM is present which may require additional therapy [1].

4.1 Clinical symptoms

The clinical manifestations of pulmonary non-tuberculous mycobacteriosis are nonspecific in HIV-negative patients with local primary diseases or systemic immunosuppression. Symptoms such as cough, sputum (sometimes bloody), dyspnea, fever, weight loss and reduction in general health may also be caused by the underlying disease and should be attributed to NTM disease only if diagnosed in accordance with the mentioned criteria and if extend of disease can be assessed (see **Table 5**). The symptoms resemble those of tuberculosis but are less pronounced, rather subacute, and cannot be assigned to NTM disease without cultural or molecular-biological species identification. Therefore, a quickest possible species diagnosis is an essential basis for treatment.

The symptoms of extrapulmonary infections caused by NTM (see **Table 3**) are related to the site of involvement [1,95].

4.2 Radiological manifestation

Radiological diagnostic investigations including controls during the course of the disease are helpful in clarifying if a disease is present which needs treatment. Two typical pulmonary radiologic manifestations can be distinguished: A mainly fibro-cavernous form, similar to tuberculosis, and a nodular/bronchiectatic form, both may however overlap, too [1]. Besides the basic chest X-ray for diagnostic evaluation, mainly high-resolution computed tomography (HRCT) is used, in particular for better detection of bronchiectasis or other structural changes as infiltrates and cavities.

Typical findings of non-tuberculous mycobacterioses include pneumonic infiltrates, thick walled cavities, bronchiectasis with surrounding infiltra, disseminated small patchy infiltrates, and lymph node enlargements (see **Fig. 2** and **Fig. 3**).

Radiological differentiation from tuberculosis is difficult, ultimately decisive is the proof of the pathogen [99,100].

One must distinguish the radiographic findings of extrinsic allergic alveolitis due to inhalation of contaminated water with MAC ("hot tub lung") [19] or due to *M. immunogenum* contaminated metalworking fluids [20].

4.3 Microbiological diagnostics and drug susceptibility testing

The detection of the microbiological pathogen from the sputum or other clinical (biopsy) specimens makes the most important contribution to the diagnosis [1,95,101]. The identification of the pathogen contributes significantly to the decision about the need for treatment (see section 5 – 8, p. 259 – 268).

Isolation of mycobacteria is still the reference standard for the microbiological diagnostics of infections caused by NTM. For certain situations (urgency, ambiguous culture results), a specific detection of NTM in the specimen can be performed using nucleic acid amplification tests (NAT) (see also 4.3.6, "Identification of mycobacteria," p. 258).

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The appendix includes selected mycobacterial species with their microbiological (and therapeutic) features (Chapter 8, Appendix 1, p. 264).

4.3.1 Clinical specimens

Due to the different locations of diseases caused by NTM a variety of specimens can be taken into account to be used for the culture of mycobacteria [1, 102]. Bronchopulmonary specimens such as sputum, bronchial secretions, bronchoalveolar lavage – in children also gastric aspirates (see Chapter 7, "Diagnostics and therapy of non-tuberculous mycobacteriosis in children," (p. 262) – can be examined in suspected pulmonary infections or diseases, as well as the biopsy material from appropriate locations (e.g., lymph nodes, skin).

It is important that tissue samples are stored in saline and not in formalin. Swabs are less suitable for diagnostics. Unless other specimens are not possible, swabs should not be transported in containers used in general microbiology (for this is an inappropriate medium), but rather in a tube with saline.

Aspirates (e.g. cerebrospinal fluid, pleural aspiration, pericardial aspiration) are used natively (i.e. without additives) for examination. Since mycobacteria are usually present in very low numbers in these samples, it is important to obtain as much volume as possible. Also, blood and bone marrow samples can be analyzed, but are only useful in patients with cellular immunodeficiency.

For accurate diagnosis of pulmonary non-tuberculous mycobacterial disease, it is important to examine multiple samples from patients [1]. Only by multiple detection of the same NTM species the diagnosis can be confirmed in non-sterile specimens (see ● **Table 5**). From primarily sterile samples, such as lymph node aspirates, multiple-detection is not required. For the diagnosis of swimming pool granuloma in case of the adequate histology, the unique isolation of *M. marinum* from a skin biopsy is sufficient.

4.3.2 Contamination of clinical specimens with NTM

Contamination of specimens with NTM may result in a misdiagnosis and should therefore be avoided. NTM are known to be widespread in aqueous environments and adhered to biofilms [8, 11, 103]. Cartridges from water treatment plants and similar water scoured units can therefore be densely colonized with NTM. For this reason NTM can be interpolated into the specimen through bronchoscopes, which have been rinsed with deionized water [68, 104].

Patients should not brush their teeth or rinse the mouth with water before sputum delivery so as not to contaminate the sputum by mycobacteria from water. This is especially important when it comes to multiple detection. Since patients generally use the same faucet on subsequent days, the same NTM species located in the biofilm at the water supply may be detected several times, hence the criterion of multiple evidence is falsely satisfied.

Buffers and solutions used for the decontamination of the specimens that have not been autoclaved may also be contaminated with NTM, and this can also compromise the patient sample in the course of processing in the laboratory. This risk, however, can be prevented through quality-assured laboratory work.

4.3.3 Smear microscopy

The microscopic detection of mycobacteria is based on the acid fastness depending on the composition of the bacterial cell walls [105]. By microscopy NTM can not readily be distinguished from TB bacteria. Some species, however, are characterized by a more coccoid morphology or a noticeable banding pattern, henceforth the microscopy in some cases may give a first indication of the presence of NTM.

For microscopic examination, two different techniques can be applied. The common method is bright-field microscopy. With this technique smears are stained with carbol fuchsin, either with the classical Ziehl-Neelsen method or the modified Kinyoun method – without heating. Alternatively, fluorescence microscopy can be performed, with the advantage of examination at lower objective magnification – and without oil immersion. With this technique smears can be analysed in a shorter amount of time, due to enlarged depth of field and field of view.

4.3.4 Nucleic acid amplification tests (NAT) for direct detection

Commercially available techniques for nucleic acid detection of mycobacteria directly in the specimen are mainly restricted to the detection of TB bacteria [106]. A negative result of such an NAT from a smear positive specimen, therefore, serves as rapid diagnostic indication of NTM. For the direct detection of NTM in specimens however, so far (almost exclusively) only 'homemade' techniques are available. Of particular importance is the molecular detection of NTM for identification of infections with non or poorly-growing mycobacteria such as *M. ulcerans* or *M. genavense* [107]. However, NAT for the detection of NTM, should not be used for screening purposes. The clinical significance of detection of nucleic acid of NTM without cultural growth (with the exception of non or poorly-growing species) is currently unclear.

4.3.5 Cultural evidence

Isolation of mycobacteria by culture is still the "gold standard" in the diagnosis of mycobacteria [106, 107]. This is true not only for TB bacteria, but also for NTM. Non-sterile specimen must be decontaminated to inactivate the more rapidly growing microbes of the normal flora. Culture has to be performed by using a combination of both liquid and solid media [108]. The former are well suited for the detection of NTM [109]. Some species, such as *M. avium*, can often be detected in liquid media within a few days [110]. Special liquid media facilitate the cultivation of mycobacteria from blood.

The culture media for mycobacterial diagnostics are routinely incubated at 36 ± 1 °C. In cases of skin samples, lymph nodes or other tissue samples from the body periphery, a second set of cultures additionally have to be incubated at 30 ± 1 °C. These samples may contain mycobacteria that prefer to grow at lower temperatures (for example, *M. abscessus, M. chelonae, M. marinum, M. ulcerans*). Also bronchopulmonary samples from patients with cystic fibrosis (CF) must be incubated in parallel at lower temperatures, as these patients often suffer from infections with *M. abscessus*. It is therefore important to pass this clinical information to the laboratory.

The cultivation time is usually six weeks for liquid media and eight weeks for solid. Prolonged incubation times may be required in special cases, such as in cases of suspected *M. genavense* or specimens that are microscopically positive but culture negative after six and eight weeks – with respect to liquid or solid media.

NTM are devided into slowly and rapidly growing species (see ● **Table 1** and Chapter 8, Appendix 1: "Microbiological and therapeutic features of selected species of mycobacteria," p. 264). Differentiation according to the growth rate, however, is based on standard culture conditions and does not relate to the detection time of mycobacteria from the clinical specimen.

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In case of growth of both TB bacteria and NTM in the cultures, the presence of NTM usually is thought to be a contamination [97, 98]. Again, multiple proofs of the presence of NTM must be demonstrated to confirm this as a very rare co-infection. If mixed cultures are not recognized they can lead to false-resistant results when performing drug susceptibility testing of the TB bacteria.

4.3.6 Identification of mycobacteria

In case of growth of acid-fast bacteria on solid or in liquid culture media, rapid differentiation between TB bacteria and NTM is required before reporting the result. The identification or exclusion of TB bacteria can be carried out very quickly by the use of commercially available molecular biological tests [101] (see also nucleic acid amplification tests [NAT]). In case of a negative result for TB bacteria identification of NTM should be performed.

There are now more than 150 validely described non-tuberculous mycobacterial species (http://www.bacterio.cict.fr/), and thus conventional methods do not allow sufficient differentiation. Detection methods based on the analysis of cell wall structures (by high performance liquid chromatography [HPLC] or Matrix Assisted Laser Desorption Ionization – Time Of Flight Mass Spectrometry [MALDI–TOF]), offer either insufficient resolution or are not yet sufficiently evaluated.

NTM can currently be determined with sufficient accuracy using molecular biological methods. There are gene probes for a few mycobacterial species, strip hybridization tests for 14 to 30 species, or the sequencing of specific genes or gene regions (for example, 16S rDNA) available.

The first commercially available test for the identification of NTM was hybridization with gene probes (AccuProbe® Gen-Probe) – for the detection of species-specific ribosomal RNA. However, this test is limited to a few species: *M. avium-complex*, *M. avium*, *M. intracellulare*, *M. kansasii*, and *M. gordonae*.

By using strip hybridization assays (GenoType CM/AS®, HAIN Life Science [111 – 113]; INNO-LiPA MYCOBACTERIA v2®, Innogenetics [114 – 115]) a variety of the most important species of mycobacteria can now be identified in a single analysis. The tests are based on the amplification and detection of gene fragments that show both conserved and variable sequence segments (23S rRNA gene or "internal transcribed spacer"). With these hybridization tests nitrocellulose strips are provided with species-specific oligonucleotides. The species are identified on the basis of the characteristic banding pattern (see **Fig. 4**). Within a few hours these tests can identify a number of important species of mycobacteria, including TB bacteria, coming from positive liquid and solid cultures (not yet sensitive enough for the application directly on clinical specimens).

The identification of the species by sequencing of specific genes is the most laborious method, but all known and unknown species can be differentiated. The method most widely established is the sequencing of the 5' region of the gene coding for the ribosomal 16S rRNA [116 – 119].

The advantage of the analysis of the 16S gene lies in the fact that these sequences are known from all mycobacterial species and also from every new validated species since this sequence has to be submitted to sequence databases prior publishing.

The identification of the species on the basis of the obtained DNA sequence is available via comparison with sequence databases, such as the National Center for Biotechnology (NCBI; http://www.ncbi.nlm.nih.gov/BLAST) or the Ribosomal Differentiation of Medical Microorganisms (RIDOM; http://www.ridom-rdna.de/). However, the final evaluation of the result requires sufficient experience, since some mycobacterial species differ by only a few bases in their respective genes. Furthermore, databases often allow uncontrolled entry of sequences leading to faulty and non-quality-controlled entries. The identification of a particular species should only be confirmed with full compliance (identity) of the sequence.

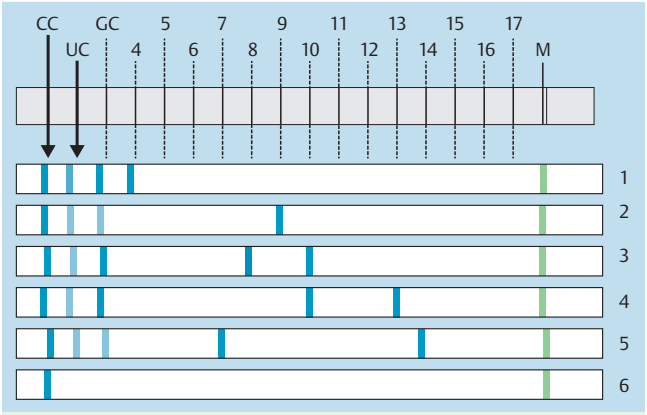


Fig. 4 Species-specific banding patterns of a strip hybridization assay for the identification of mycobacteria (GenoType® CM/AS). CC: conjugate; UC: universal control, GC: genus-specific control for mycobacteria; 4 – 17: species specific bands, M: marker of the upper side of the strip. Lane 1: *M. avium*; lane 2: *M. intracellulare*; lane 3: *M. gordonae*; lane 4: *M. malmoense*; lane 5: *M. fortuitum*; lane 6: negative control. [Courtesy of Dr. Elvira Richter].

4.3.7 Validation

All results obtained using molecular biological techniques should be reviewed for plausibility even after reporting. This can be done by analyzing certain physiological characteristics of mycobacteria (for example, colony morphology, growth rate, temperature preference, pigment formation, photochromogenicity) [101]. With these features the identification of certain NTM species can be confirmed.

Rapidly and slowly growing mycobacteria can be distinguished by the growth rate. *M. marinum* and *M. kansasii* are photochromogenic species that produce a yellow pigment after light exposure, whereas *M. gordonae* or *M. szulgai* are pigmented already in the dark. *M. marinum*, and *M. malmoense* grow preferably at about 30 °C, less at 37 °C, especially on solid media.

4.3.8 Drug susceptibility testing

There is currently no standard method available for drug susceptibility testing of NTM. It should be noted that the results of *in vitro* tests are often not corresponding with the in-vivo efficacy [1]. In the guidelines of the CSLI (Clinical and Laboratory Standards Institute) [120] the methods described for susceptibilty testing of *M. avium, M. kansasii, M. marinum* and rapidly growing mycobacteria are no longer used (working with the radiolabeled media BACTEC 460-system), or are not evaluated (in the microtiter plate-based liquid medium method), so that both methods are not routinely carried out in Germany. Some specialized laboratories perform in case of specific indications, drug susceptibility testing in liquid or on solid media analogous to the methods proposed by the CLSI.

A recent liquid culture system – the BACTEC MGIT 960 (Becton-Dickinson), for which drug susceptibility testing for *M. tuberculosis* is established worldwide [121, 122], may be used as a substitute for the BACTEC 460 system for testing – of slowly growing mycobacteria although no clinical evaluation has been performed

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so far [123,124]. Only a limited number of drugs have been included.

The standard drug susceptibility testing method for *M. tuberculosis* is not performed as estimation of the minimal inhibitory concentration (MIC). In contrast, the methods generally accepted are based on growth of TB bacteria on solid or in liquid media containing a certain concentration of the drug ('critical concentration') [125].

Performing drug susceptibility testing using the critical concentrations established for TB bacteria, NTM often have species-characteristic patterns of resistance to various antibiotics. Many NTM are resistent to INH, for example, *M. kansasii, M. malmoense, M. szulgai* or *M. marinum*. Nevertheless INH for the treatment of *M. kansasii* is often recommended [1] reasoned by the argument of a low level resistance that is compensated by the serum concentration of INH. Also for other antibiotics the MICs of NTM are higher than the concentrations used for drug susceptibility testing of *M. tuberculosis*, such as RMP in *M. avium*. So far, however, is little experience with MIC determination in mycobacteria as well as judging these results with regard to the therapy. It should be emphasized again that for NTM often a discrepancy is observed between the results of *in vitro* tests and the success of treatment [1].

For drug susceptibility testing of rapidly growing species the BACTEC MGIT 960 method can not be used – currently. Liquid medium-based methods with manual evaluation are used in some cases for certain issues. The observation that some *M. abscessus* strains start to grow with a prolonged incubation time in the presence of clarithromycin, and furthermore the genetic evidence of the gene involved, led to the conclusion that certain *M. abscessus* strains are characterized by an inducible clarithromycin resistance [126,127]. The clinical relevance of this observation is not yet clear. Overall, the development of resistance during therapy is rarely observed with NTM [128].

For the implementation of molecular methods for drug susceptibility testing reliable phenotypic methods are necessary to be able to correlate specific mutations with resistance. To date, molecular studies include mainly the mutation analyzes of the 23S rRNA gene in *M. avium-complex* and rapidly growing mycobacteria, and the erm gene in *M. abscessus*, to correlate with macrolide resistance [126,127,129].

The determination of serum concentrations could be of more importance in the future because of certain interactions of drug combinations and their associated variables and reactions [130]. Serum levels due to potential drug interactions could be to low for example, but tissue levels – which are not routinely measured – may be of even greater importance [131,132].

5 Treatment of non-tuberculous mycobacteriosis in HIV-negative patients

▼

In addition to the correct identification of the species, the need for treatment depends on semi-quantitative microbiological criteria, as defined in 2007 by ATS and IDSA [1]. The more numerously seen in smear microscopy and the more frequently found in sputum (hence the higher the number of bacteria) the more likely is the need for treatment (see **Table 5**). Smear-negative and exclusively culture-positive cases are assumed to have a low mycobacterial load and therefore require a more careful evaluation of the need for therapy. The same holds true for the exclusive proof from bronchoscopic materials in patients with smear and culture negative or none available sputum. The need for treatment can be also indicated, (even with a low bacterial count) when a clearly pathologically and radiologically progressive disease is present, e.g. in form of a thick-walled, thus freshly inflamed cavity or disseminated infiltrates. In the presence of a non-tuberculous mycobacteriosis with no obvious local predisposing lung disease ("terrain factor" [61] one should consider treating also an only circumscribed disease process (see **Fig. 2**), because such a manifestation resembles pathophysiologically more an acute tuberculosis; even in the theoretically possible case of a spontaneous remission, such a form of a mycobacteriosis could lead to persisting structural damage. However in non-tuberculous mycobacteriosis with an existing terrain factor, colonization must be delineated more thoroughly from disease for which treatment is needed (see **Table 2**).

Special care is required in the diagnosis of NTM disease because of the associated potential risks in drug and/or surgical therapy [16,133]. Treatment costs can also be significant, similar to those for HIV infection [134].

5.1 Pharmacotherapy

Since antimycobacterial therapy of non-tuberculous mycobacteriosis may be associated with significant adverse drug effects and since its duration usually, according to the present recommendations, exceeds that of antituberculous therapy, there is a high demand of acceptance from the side of the patient. Moreover, there is often an underlying chronic disease present which often already has caused a limitation of the patient's functional reserves, whereby additional symptoms due to the non-tuberculous mycobacteriosis may be experienced as less alarming. However, even without severe functional limitations due to the underlying disease the symptoms are less pronounced because the pathogenicity of most NTM species is lower than in tuberculosis (which on principle always requires treatment) of comparable extent what may diminish the acceptance and the psychological strain of the disease by the patient [45].

Such circumstances may explain why some patients are reluctant to follow the recommendation of long-term antimycobacterial therapy in addition to potentially complex therapy for the underlying disease. The relatively none-pronounced symptoms should be taken into account by doctors when establishing a therapeutic concept, in particular when only limited efficacy of therapy is likely. This is particularly true for patients with advanced lung diseases. Though, the fear of a proceeding destruction of the lungs caused by non-tuberculous mycobacteriosis may be an indication for treatment. However, there must be an awareness that the indication for a – presumably palliative – treatment may be in a 'gray area' of experience and individual observation. Little is known from the literature of the long-term natural history (ten years and longer) of NTM disease after diagnosis, both, with or without treatment.

The basic principles of prescribing a conservative pharmacotherapy are similar to those in tuberculosis. Characteristics of antibiotic therapy in selected NTM species are described in Chapter 8 (Appendix 1: "Microbiological and therapeutic features of selected species of mycobacteria," p. 264). Combination therapy over a prolonged period is almost always recommended for NTM disease, as monotherapy carries the risk of rapidly developing resistance [128].

In Chapter 9 (Appendix 2: "Properties of the drugs used in the treatment of NTM disease," p. 268), the properties (mechanism of action, pharmacogenetics, dosage, contraindications and side

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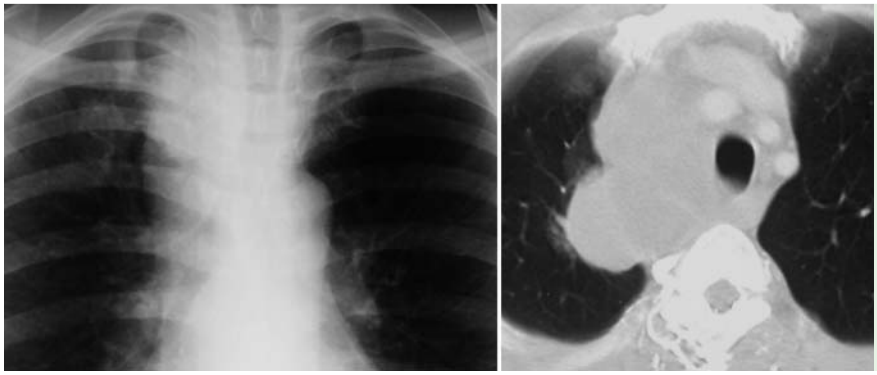


Fig. 5 Chest X-ray and CT of a 35-year-old HIV-positive patient in stage AIDS with a history of cerebral toxoplasmosis. Eight weeks after initiation of antiretroviral therapy pronounced right-sided mediastinal lymphadenopathy with detection of *M. avium-complex* on PCR. [Courtesy of Department of Radiology and Nuclear Medicine, University Hospital Schleswig-Holstein, Campus Lübeck].

effects) of drugs used in the treatment of NTM disease are briefly summarized in alphabetical order. Further details can be found in the recommendations of the DZK and the DGP for the treatment of tuberculosis [135].

5.2 Surgical therapy

Surgical therapy should in principle be performed in curative intention. With pulmonary non-tuberculous mycobacteriosis, resection of the diseased part of the lung should be considered if the course under drug therapy is unlikely to result in a permanent improvement of the disease manifestations of the patient [59, 96, 136]. Furthermore, the indication for resection of a residual cavity or an infected destroyed lung region should be checked if hereby other complications are caused such as infections with bacteria (*P. aeruginosa* and others) or fungi (*Aspergillus spp.*) with recurrent pneumonia, hemoptysis or advancing chronic obstructive bronchitis with or without bronchiectasis.

In patients with only mild or lacking underlying disease, patients should be at first observed for longer periods after a clinically, radiologically and microbiologically confirmed successful therapy, even if a residual lesion should be present since; there is little data on long-term histories. In patients with clinically apparent functional limitations imposed by the underlying disease, the indication for resection in terms of functional operability must be carefully assessed since the frequency of complications is higher than in post-tuberculous residuals [137]. In addition, clinical decision can be difficult because the long-term prognosis of the patient is often more determinded by the underlying disease or the operation itself, rather than by the non-tuberculous mycobacteriosis [59, 138].

Deep skin and soft tissue infections caused by *M. marinum* may also require surgical intervention (debridement), but only in combination with antibiotic therapy [1]. Similar approaches can be assumed for skin and soft tissue infections caused by *M. xenopi*, *M. fortuitum* or other NTM species, wherein Buruli ulcers thermal therapy is described as an additional option [139].

The indications for surgery in pediatric lymphadenitis are described in the relevant sections in Chapter 7, "Diagnosis and therapy of non-tuberculous mycobacteriosis in children," p. 262.

5.3 Treatment of underlying disease

The underlying disease should always be treated as proposed by Radenbach already in the 1970s [40] and lastly included explicitly into the ATS/IDSA guidelines [1]. As well-proven in patients with cystic fibrosis, in patients with COPD, too, – with or without bronchiectasis – therapy according to the guidelines should improve the prognosis independent on the treatment of the mycobacteriosis. It is readily plausible to assume that an improvement of bronchial clearance alleviates the terrain factor being for the colonization and the local multiplication of the mycobacteria [140].

Unfortunately, previous studies do not or rarely take into account the interpretation of chemotherapy results for the question of whether or not an adequate treatment of the underlying disease was performed. Thus, the influence of such measures (e.g. anti-obstructive treatment, physiotherapy) can only be assessed from a clinical perspective.

6 Non-tuberculous mycobacteriosis in HIV infection

6.1 Clinical presentation

The epidemiology and clinical presentation of non-tuberculous mycobacteriosis in patients with HIV infection has a number of unique features. Depending on the cellular immune status, disseminated and localized pulmonary forms of the disease can be defined. Disseminated forms as a late manifestation of severe immunodeficiency (CD4+ lymphocyte count < 50/µl) are among the most distinct AIDS-defining illnesses. MAC (*Mycobacterium avium-complex*) is found most commonly, among those *M. avium* is responsible for 95 % of NTM infections in this setting [1].

The ubiquitous common pathogens are transmitted either by inhalation or the gastrointestinal route, and there are no known factors to increase the risk for transmission [76]. Transmission from patient to patient has not been documented. In patients without effective antiretroviral therapy and without chemoprophylaxis, the incidence of MAC is 20 – 40 % over a period of two years [141, 142]. The clinical presentation is characterized by the generalised symptoms of fever, night sweats, weight loss and fatigue. Key signs include lymphadenopathy and hepatosplenomegaly. The lungs may be also affected.

In the era of effective antiretroviral therapy, disseminated MAC infection is rarely observed. It occurs in untreated AIDS patients with severe immune deficiency, but in Western countries, increasingly only localized manifestations are observed, such as focal lymphadenitis (**Fig. 5**), pulmonary infiltrates, and (rarely) osteomyelitis. These forms occur more frequently after initiation of antiretroviral therapy as a result of the Immune Reconstitution Inflammatory Syndrome (IRIS) (see below) [143].

In the context of HIV infection, limited pulmonary non-tuberculous mycobacteria and lymphadenitis can be expected with otherwise moderate immune deficiency (see **Fig. 6**), at CD4+ lymphocyte counts between 200 – 500/µl [144]. A common pathogen is *M. kansasii*.

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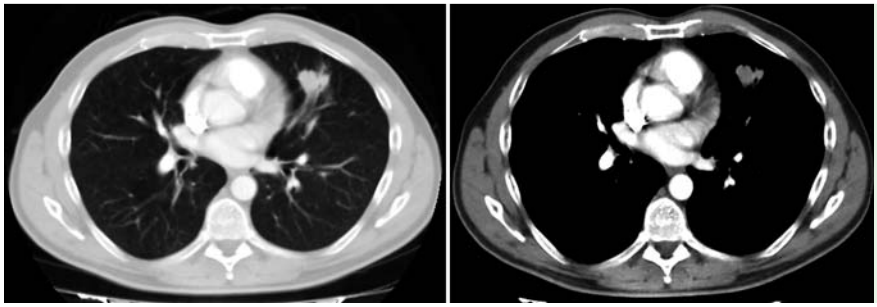


Fig. 6 HIV-positive patient with hemoptysis and inhomogeneous infiltrate in the lingula: biopsy epithelioid cell granulomatosis, detection of *M. avium* in culture. [Courtesy of Department of Radiology and Nuclear Medicine, University Hospital Schleswig-Holstein, Campus Lübeck].

A study conducted in the 1990s demonstrated an incidence of 115/100,000 HIV-positive patients per year, which is roughly 150 times higher than in the normal population [145].
In predominantly localised disease, peripheral infiltrates are particulary emphasized in the middle and lower lobes, less common are cavitations which have a less favourable prognosis [146].
Besides MAC and *M. kansasii* a variety of other NTM are described in HIV infection, such as *M. celatum*, M. *genavense*, *M. haemophilum*, *M. malmoense*, *M. simiae* and *M. xenopi*. As the number of cases are small, very few or no studies exist.
An important complication of therapy is the Immune Reconstitution Inflammatory Syndrome (IRIS) [147]. It occurs with a rapid rise in CD4+ lymphocytes after initiation of antiretroviral therapy and emerges in MAC infection at a similar rate compared to tuberculosis. There is a mismatch between distinct cellular infiltrates and paucibacillary tuberculosis infection [148], and thus a microbiological diagnosis is difficult to prove. Blood cultures are commonly negative. Clinically, fever and focal lung and lymph node disease are prominent [143]. With antimycobacterial therapy, the course of IRIS can be either self-limiting or progressive, and, similarly to tuberculosis, a small proportion of patients require in addition anti-inflammatory therapy with glucocorticosteroids [147, 149].

6.2 Diagnosis

The diagnosis of disseminated MAC infection is confirmed by blood cultures in more than 90% of cases [1]. Frequently, the pathogen can be isolated from respiratory materials. In unclear cases, lymph node biopsies or bone marrow aspirates should be performed. Laboratory tests may reveal an increase in alkaline phosphatase and normocytic anaemia [150].
Microbiological detection methods have a relatively high sensitivity and specificity for the detection of localized NTM infections in HIV-infected patients. In a population-based study, 41% of *M. kansasii* – mostly pulmonary – disease was sputum smear positive. Cultural pathogen detection had a high positive predictive value for clinically relevant infection: 85.7% in HIV-positive patients and 71.4% for HIV-negative [145]
A mycobacterial PCR can accelerate the differentiation of *M. tuberculosis-complex* from NTM in patients with smear positive samples.

6.3 Therapy

Treatment recommendations are generally the same for HIV-infected patients with NTM as in Chapter 8 (Appendix 1: "Microbiological and therapeutic features of selected species of mycobacteria," p. 264). In the following, some special features are listed. With disseminated MAC infection, daily combination therapy with at least two substances is recommended [76]. Clarithromycin and Ethambutol have been most extensively studied [151]. The standard dose of clarithromycin (1000 mg/d) should not be exceeded, as higher doses have been associated with increased mortality [152]. Azithromycin may be used as an alternative option although less data are available from studies on the efficiency of this substance [153].
The benefit of a triple combination regimen containing rifabutin was not consistent in two controlled trials of HIV-infected patients in terms of survival, and must be weighed against a higher rate of side effects. However, triple drug combinations have been shown to reduce the emergence of macrolide resistance during therapy [152]. The use of the third drug should be considered for extensive disease, high mycobacterial loads, and unavailability of effective antiretroviral therapy [76].
Rifabutin requires dose adjustment in patients receiving protease inhibitors for the treatment of HIV infection. Also, during therapy with non-nucleoside reverse transcriptase inhibitors (NNRTI) rifabutin should only be administered when patient and physician(s) can ensure therapeutic drug monitoring with dose adjustment if necessary [135]. Therapy should be in collaboration with a physician experienced in the treatment of HIV-positive patients. Other alternatives include the aminoglycosides streptomycin and amikacin, but with the disadvantage of parenteral administration. As recommended in international guidelines for tuberculosis, antiretroviral therapy should start at least two weeks after the initiation of anti-mycobacterial therapy to reduce manifestations and of the immune reconstitution syndrome (IRIS) (see 6.1, "Clinical presentation," p. 260) [76].
According to current evidence the duration of therapy should be at least twelve months after culture conversion. In disseminated MAC disease success of treatment is clinically monitored by blood culture, performed 2 – 4 weeks after initiation of therapy. In pulmonary infections, microbiological assessment of sputum, or in exceptional cases other respiratory samples, is carried out after an initially positive result. In case of treatment failure in terms of a lack of clinical and radiographic improvement, patient's compliance should be evaluated in the first place. A change in therapy should include at least two new drugs, as mycobacteria have a low threshold for clarithromycin resistance [150]. However, there are no controlled studies on the effectiveness of this approach. Fluoroquinolones and aminoglycosides can be considered [76]. Of central importance in the treatment of MAC infection is the immune status of the patient. This should always be assessed to determine whether optimization of antiretroviral therapy is required.
For the treatment of *M. kansasii* infection in HIV-infected patients, the same recommendations apply as for HIV-negative patients: Triple therapy with isoniazid, rifampicin and ethambutol (see Chapter 8, Appendix 1: "Microbiological and therapeutic features of selected species of mycobacteria," p. 264). However, the replacement of rifampicin by rifabutin should be considered

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in individuals receiving concomitant antiretroviral therapy. Alternatively, it is recommended to replace the rifamycins with a macrolide or moxifloxacin [1].
IRIS has already been discussed as a possible complication of therapy (see section 6.1, "Clinical presentation," p. 260) [147 – 149].

6.4 Prevention

Primary prophylaxis in HIV-positive patients with a poor immune status (CD4+ lymphocyte count < 50/µl) is effective, but taking into account incidence and pill burden, it is not generally recommended, because a sufficient immune reconstitution is usually achieved by way of effective antiretroviral therapy [76].
The use of primary prophylaxis for NTM is best discussed in patients with immune deficiency that cannot be improved by antiretroviral therapy. Controlled trials have demonstrated a significantly reduced risk of NTM infection through the use of the macrolide antibiotics azithromycin and clarithromycin for primary prophylaxis [154, 155]. For this indication azithromycin has the advantage of once-weekly dosing. Possible long-term side effects of these substances have to be observed.
Because of the high risk of recurrence after disseminated MAC infection, maintenance therapy is required until the CD4+ cell count exceeds the threshold of ≥ 100 cells/µl for a period of 3 – 6 months on antiretroviral therapy [156, 157]. Standard regimen is the dual combination of macrolide and ethambutol. Secondary prevention after localized infections is unnecessary in most cases and its use has not been established by clinical studies.

7 Diagnosis and therapy of non-tuberculous mycobacteriosis in children

Diseases caused by non-tuberculous mycobacteria in children show some peculiarities. Therefore, this is dealt with here in a separate section. The chapter is based on a 2005 article [158] supplemented by current data.

7.1 Epidemiology

International studies showed an annual incidence of diseases caused by NTM ranging from 0.8 to 4.5 per 100,000 children [159 – 162].
In Germany the epidemiology of non-tuberculous mycobacteriosis was first studied nationwide in immunocompetent children from October 2002 to September 2005 [163]. In this prospective study by the Robert Koch Institute (RKI) and the surveillance unit for rare pediatric diseases in Germany (ESPED) a network of voluntarily participating children's hospitals collected demographic and clinical data on 102 children with laboratory-confirmed NTM disease and no prior history of NTM disease. During the same period of time, a nationwide laboratory sentinel network reported NTM isolates. Based on these two data sources, an annual incidence of 1.3 per 100,000 children could be calculated, taking into account the estimated number of cases not detected in both systems. 97 % of children had a localized lymphadenitis and 93 % were less than five years old. This chapter therefore focuses on the diagnosis and treatment of lymphadenitis caused by NTMs.

7.2 Spectrum of pathogens and clinical manifestations

Whether or not infection with NTM in children leads to a clinical disease depends on the virulence of the pathogen and host intrinsic factors, such as immune status and age. The nature of the source of infection and exposure also plays a role. ● **Table 6** shows typical clinical pictures and frequently detected species in children. In contrast to lymphadenitis, a specific disposition such as a cellular immunodeficiency (e.g. HIV infection), interferon-gamma (INF-γ), interleukin-12 (IL-12) receptor defects [164], STAT1 defect, septic granulomatosis CGD [165], cystic fibrosis [166, 167] (● **Table 4**), the state after surgery [168] or invasive examination play a role if other organs are infected or a generalized disease occurs.
The most common clinical manifestation of disease in immune competent children is cervical lymphadenitis (● **Fig. 7**). Prospective studies from Germany, the Netherlands and Australia unanimously show that the main causative agent is *M. avium-complex* [158, 160, 163]. The German study also sporadically detected *M. kansasii, M. celatum, M. chelonae* and *M. malmoense*.

Table 6 Diseases caused by non-tuberculous mycobacteria, and often isolated pathogens in children (adapted from [170]).

Disease	Main pathogens
Generalized disease (with immunosuppression or genetic predisposition)	*M. avium-complex*
Skin and soft tissue infections	*M. marinum, M. ulcerans, M. chelonae, M. abscessus*
Catheter-associated infections	*M. abscessus, M. chelonae*
Lymphadenitis	*M. avium-complex, M. malmoense, M. scrofulaceum, M. haemophilum*
Otitis media, mastoiditis	*M. abscessus, M. kansasii, M. xenopi*
Pulmonary infections	*M. avium-complex, M. kansasii, M. xenopi*
Cystic fibrosis (pulmonary infections)	*M. abscessus, M. avium, M. chelonae*

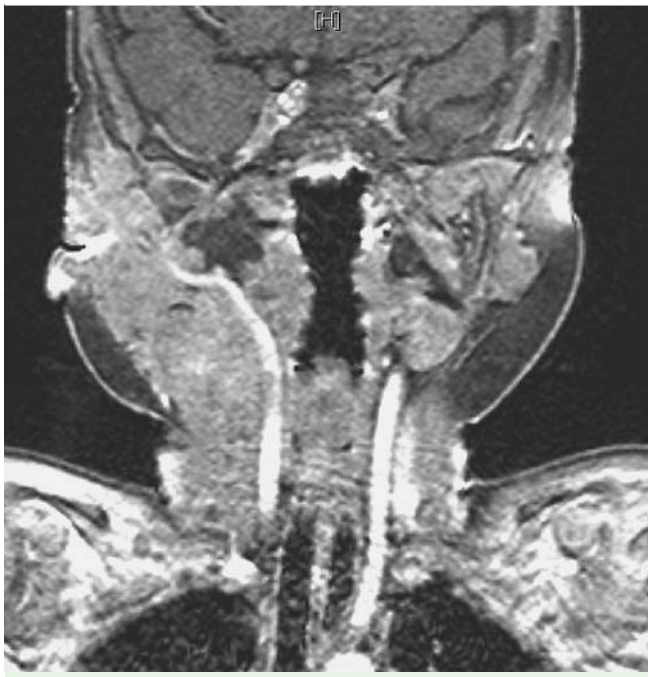


Fig. 7 Non-tuberculous mycobacteriosis by *M. avium*, 15-month-old female child – mainly in the lymph nodes of the right side of the neck (MRI). [Courtesy of Dr. Roland C. Bittner, Department of Diagnostic and Interventional Radiology, Helios Klinikum Emil von Behring, and Dr. Michael Barker, Department of Pediatric and Adolescent Medicine, Helios Klinikum Emil von Behring].

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Table 7 Typical clinical features of non-tuberculous mycobacterial lymphadenitis in children [171, 172].

unilateral swelling of lymph nodes in the jaw angle, pre-auricular (in the region of the parotid gland) or submandibular
bluish-red color, possibly duct fistula
movable lymph nodes
firm consistency without fluctuation
tenderness to palpation

It mainly affects children under the age of five. Frequency of individual NTM species is dependent on the geographical location (see "Epidemiology"). For example, *M. malmoense* is rare in the United States, but is often isolated in Europe [169].

The medical history and clinical examination indicate whether a lymphadenitis is caused by NTM. **Table 7** shows the characteristics of the typical clinical picture [171, 172].

Diagnosis of cervical lymphadenitis is supported by a healthy disposition of children without systemic symptoms such as high fever, by lack of response to non-specific antibiotics and a course of disease for more than two weeks.

7.3 Diagnosis

For the differential diagnosis, history of exposure risk, country of birth and/or nationality of the child are of importance. Close contact with people with infectious tuberculosis, and/or the origin of high-prevalence-tuberculosis regions such as the states of the former Soviet Union, Southeast Asia or Southern Africa indicate a TB diagnosis.

The tuberculin skin test with two units of tuberculin PPD RT 23 may also be false-positive after contact with NTM, since the antigens used in the test may also be present in some NTM species (cross-reactivity). In the German study of RKI and ESPED 82 % of the children had an induration of more than 5 mm; and nearly 20 % more than 15 mm [163] .

A positive tuberculin skin test may generally be regarded as an indication of mycobacterial genesis (also as a result of BCG vaccination). However, it does not permit differentiation between infection and/or disease caused by *M. tuberculosis* and NTM. In most cases an interferon-γ test (IGRA) is helpful due to the higher specificity for *M. tuberculosis* [173, 174]. Cross-reactions with false positive results in the IGRA are only possible for *M. kansasii, M. marinum, M. flavescens* and *M. szulgai* [93, 94]. Pre-existing BCG vaccination does not lead to a positive test result, in contrast to the skin test. While tuberculosis is unlikely with a positive tuberculin skin test and a negative IGRA, disease caused by NTM is even more likely.

Chest X-ray is part of the differential diagnosis. In immunocompetent patients with mycobacterial lymphadenitis by NTM the chest image is almost always normal, whereas in patients with lymph node tuberculosis there may be concomitant pulmonary disease [175]. A sonographic examination provides further diagnostic information and serves as a support in the planning of the (mostly) surgical therapy. Small calcifications in the lymph node may indicate a previous mycobacterial infection [176].

Detection of NTM by molecular genetic methods or in culture in the otherwise sterile material obtained by fine needle aspiration can contribute to the diagnosis.

During the extirpation of the affected lymph node one should also obtain unfixed tissue for the pathogen diagnosis and possibly for special histopathological staining (when a tumor is suspected). Incision and drainage is not recommended as this usually leads to the formation of a sinus tract with chronic discharge [177]. Even the unique detection of NTM from sterile lymph node tissue is conclusive for the diagnosis. In the case of disseminated disease an immunological diagnostics should be made in terms of an underlying disease.

7.4 Therapy

Although lymphadenitis by NTM is not a viable threat to the immunocompetent child, it may result in a significant burden of disease due to the destructive local process, the chronic nature of the disease, and to long-term complications such as formation of fistula [178].

The standard treatment of cervical lymphadenitis due to NTM is surgery. The evidence is based on a thirty year-old study from Texas [179]. In this first large case series of 82 cases of lymphadenitis due to NTM, and a further 298 cases from the literature, 92 % of cases were cured by surgical removal of the macroscopically affected lymph nodes alone. There was no significant difference compared to the cure rate of 95 % in those cases with additional antimycobacterial combination therapy

In contrast, the sole approach of incision and drainage without removal of the pathologically altered lymph nodes led to a cure rate of 16 % [179]. The higher cure rates of surgery compared to an antimycobacterial combination therapy are confirmed by current randomized controlled trials [162, 180].

In the prospective randomized study by Lindeboom et al. sole surgical treatment was compared with a combination therapy of clarithromycin and rifabutin over a period of at least twelve weeks [180]. There were fifty children per group, with cervicofacial lymphadenitis by NTM (*M. avium* in 71 cases and *M. haemophilum* in 22 cases). The cure rates after surgical therapy were 96 % after three and six months.

After treatment with antimycobacterial drugs, 44 % were cured after three months with a further 32 % having regression of lymph node enlargement. 66 % was the proportion of successfully treated patients after six months. 28 % had complications following surgical therapy. 74 % of children treated with antimycobacterial drugs were reported to have had adverse drug reactions, with two children reported to have had serious adverse drug reactions [180]. The prospective study in 2009 by Blyth et al. showed similar results: 94 % cure rates after complete surgical excision and 70 % under the sole drug therapy (macrolide monotherapy or combination therapy with macrolides and rifampicin) [162].

With regards to vascularization, skin thickness and skin surface in the affected region, the cosmetic result was estimated to be better after one year for the group of children undergoing surgical therapy [181].

Macroscopically affected lymph nodes and (possible) existing fistula should be removed as completely as possible – incision of melting lymphnodes resulting in draining of puss in the wound needs to be avoided [182]. Though surgery is technically challenging due to the proximity of important anatomical structures, complications (particularly a compression or damage to branches of the facial nerve) are rare when surgery is performed by an experienced surgeon [181].

Although all affected tissue can not always be removed in this procedure, recurrences are rather rare. Enlarged and suspect lymph nodes on the opposite side of the main affected area are often found in the preoperative imaging but usually disease progression does not occur on the other side after a unilateral surgery on the affected side [181].

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Table 8 Drug therapy in immunocompetent children with isolated NTM lymphadenitis due to *M. avium-complex* [170].

Clarithromycin 15 – 30 mg/kg/day in divided doses p. o. (maximum daily dose, see manufacturers product information) or azithromycin 10 – 12 mg/kg/day (maximum daily dose, see manufacturers product information)
Rifampicin 350 mg/m² body surface, max. 600 mg/day (i. e.: 0 – 5 years: 15 mg/kg day, 6 – 9 years: 12 mg/kg day, 10 – 14 years: 10 mg/kg day) or rifabutin 5* mg/kg day, max. 300 mg/day
Ethambutol 850 mg/m² body surface, max. 1.75 g/day (0 – 5 years: 30 mg/kg day, > 5 years: 25 mg/kg day)

* Rifabutin is currently not approved in Germany for the child's age. The dosage information is not evidence-based. For toxicity and adverse effects see drug information of the manufacturers.

Other suspicious lymph nodes are often noticed post-operative by palpation or ultrasonography. According to experience a wait-and-see approach with regular sonographic follow-up is sufficient in these cases. Only with clear clinical and/or sonographic evidence of melting, formation of fistulas etc., is a renewed surgical intervention indicated. Relapses often occur within the first three months, but in some cases even years after the procedure [178, 183].

For a relapse the same recommendations for treatment apply as per the primary disease. In case of a relapse with formation of a draining fistula or with lymph nodes that are difficult to access surgically, medication alone or surgical procedure combined with medication can be considered [1, 170].

Medical treatment of lymphadenitis due to *M. avium-complex* and other slow-growing NTM must be a combination therapy, since monotherapy is not an option due to rapid development of resistance. Based on case series, empirical data and laboratory investigations a combination of clarithromycin or azithromycin, ethambutol, and a rifamycin derivative (usually rifampicin) is recommended (see **Table 8**).

In case of a rare intermediate macrolide resistance of the pathogen *in vitro*, clarithromycin (or azithromycin) should be part of combination therapy and the course of therapy should be closely monitored [1].

A suspected superiority of rifabutin over rifampicin by way of pharmacokinetic data obtained *in vitro* is not substantiated by clinical application.

Rifabutin is still not approved for children in Germany. There is no evidence-based data on dosing in children. Therefore and due to the narrow limits of toxicity and more frequent complications in Rifabutin administration, rifampicin should be used primarily in combination therapy. An adjunct therapy with ethambutol to counteract emerging resistance is reasonable with regular examinations (four weeks) of color vision to detect optic neuritis. For possible side effects of RMP and EMB, see Chapter 9 (Appendix 2, p. 268) and Schaberg et al. [135].

The same treatment approach can be applied in disease caused by other slow-growing NTM such as *M. haemophilum, M. kansasii, M. malmoense* and *M. scrofulaceum*, where *in vitro* sensitivity to the drugs can be highly variable (depending on the species and strain of the pathogen) and where data for clinical efficacy (and duration of therapy) is largely lacking. The duration of therapy should be at least six months, but longer therapy may be required in some cases, depending on the clinical course [170].

A combination drug therapy is primarily prescribed in a pulmonary or generalized disease. Rapidly growing mycobacteria (e. g. *M. chelonae* and *M. abscessus*) should be treated with combination therapy according to the recommendations of ATS/IDSA [1] (see also Chapter 8, Appendix 1, p. 264).

8 Appendix 1: Microbiological and therapeutic features of selected species of mycobacteria

Rapidly and slowly growing NTM are distinguished based on their growth rates under standard culture techniques (see **Table 1**). This appendix describes microbiological and therapeutic characteristics of seleted clinically very relevant species of mycobacteria in both groups (see also Chapter 6, "Non-tuberculous mycobacteriosis in HIV infection," p. 260 and Chapter 7, "Diagnosis and therapy of non-tuberculous mycobacteriosis in children," p. 262).

8.1 Slowly growing mycobacteria

8.1.1 *M. avium-complex* (MAC)

Microbiology: *M. avium-complex* (MAC) includes the two species *M. avium* and *M. intracellulare* (MAI), which are among the most important and most widespread pathogenic NTM. By the use of biochemical and morphological techniques the species hardly can be discriminated. A medical necessity to distinguish these species more accurately did not seem necessary for a long time, as management of these patients appeared indistinctive. Meanwhile significant genetic differences between *M. avium* and *M. intracellulare* have now been identified. The impact of the observed genetic differences on infectivity and clinical disease is still largely unknown. However, a large Korean study has shown that patients with *M. intracellulare* developed more severe lung disease (with a poorer response to therapy) than with *M. avium* [184].

By now, strains with genetic variabilities once considered a part of *M. intracellulare* are desrcibed as distinct species (*M. chimaera, M. colombiense, M. arosiense, M. vulneris*) [185 – 188], though possible differences in clinical relevance still must be clarified.

Based on the physiological characteristics of *M. avium* in 1990 three subspecies were distinguished (*M. avium avium, M. avium paratuberculosis* and *M. avium silvaticum*) [189]. This differentiation was confirmed by modern genetic analyses. Furthermore a fourth subspecies (*M. avium hominissuis*) was proposed, that was mainly isolated from human specimens, but included also strains isolated from pigs [190].

M. avium avium is typically detected in birds [191], with the closely related *M. avium silvaticum* detected primarily in wood pigeons [189]. *M. avium paratuberculosis* is the infectious agent of "Johne's disease" in ruminants [192]. *M. avium paratuberculosis* is usually strictly restricted to its host and does not normally occur in the environment. However, infected cows can excrete enormous amounts of organisms and thus infect other animals. The causal involvement of *M. avium paratuberculosis* in Crohn's disease in humans is controversially discussed and is not yet fully understood [193]. *M. avium hominissuis* is found primarily in humans, but also in pigs and in environmental samples. These results thus argue against the hypothesis that infection with *M. avium* in humans can be transferred only by birds. *M. avium hominissuis* is genetically highly variable. The influence of this variability on pathogenicity or virulence of different strains is the subject of current research [36, 111, 114, 115, 194].

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MAC strains are common in the environment, for example in tap water. It is therefore critical that the clinical significance of MAC recovered especially from bronchopulmonary samples be carefully assessed [68,195].

Therapy: For clinically severe disease ATS/IDSA [1] recommend treatment with the following daily regimens (**Table 9**).

For moderately severe clinical disease (nodular and bronchiectatic radiological manifestations) ATS/IDSA [1] however recommend intermittent therapy three times weekly with the following drugs (**Table 10**).

However, the efficacy of an intermittent therapy three times weekly which the ATS/IDSA guidelines recommend for the nodular/bronchiectatic form and in case of intolerance to daily dosing or in patients with less extended disease is not proven [196]. Generally, intermittent therapy for tuberculosis is not recommended in Germany [135]. Therefore, it is recommended in Germany, too, to consider such intermittent treatment variations as an alternative only in case of adverse drug reactions and taking into account the individual course of the disease under a daily therapy with three drugs. Likewise not proven is the ATS recommendation for treatment with EMB in a high dose of 25 mg/kg body weight for intermittent therapy, while for daily treatment the usual dose of 15 mg/kg is recommended. The *in vitro* MIC values of ethambutol for *M. avium* strains are highly variable and are not correlated with treatment outcomes [197]. In Germany the usual daily dose of ethambutol (15 mg/kg body weight) for adults is considered reliable. The during the 1990s recommended treatment with rifabutin instead of rifampicin could finally not convince because the rate of adverse effects of rifabutin (mainly uveitis and leukopenia) in combination therapy is high and the clinical superiority could not be substantiated, despite higher tissue concentrations and at the same time lower inhibitory concentrations *in vitro* compared to RMP [1]. One exception is concomitant antiretroviral and antimycobacterial therapy in AIDS patients, where rifabutin is preferred due to pharmacological interactions between certain antiretrovirals and rifamycins [198] (see Chapter 6, "Non-tuberculous mycobacteriosis in HIV infection," p. 260).

The addition of streptomycin/amikacin as a fourth drug may be considered in severe and extensive cavitary *M. avium* infections for more rapid reduction in bacterial load [1,199]. In case of intolerance to other drugs or macrolide resistance, further antibiotics may be included such as moxifloxacin, (although it has shown certain antagonism to clarithromycin in animal studies [200] and protionamide for which only clinical experience data exist as for the closely related ethionamide. Clofazimine is also considered as an alternative [140].

The duration of treatment depends – in case of good clinical response – among others on microbiological results. The aim should be to continue treatment at least for 12 months until sputum cultures remain negative. This typically results in 18 months of therapy [1].

8.1.2 *M. celatum*

Microbiology: *M. celatum* belongs to the 'newer' species of NTM [201]. The species was initially described mainly in HIV patients [202,203], but has also been identified as the causative agent of pulmonary infections in non-immunosuppressed patients [204]. Three different 16S rRNA "sequence types" were described, wherein the sequences of type 1 and type 3 are very similar. The sequence of type 2 differs significantly from the sequences of types 1 and 3. *M. celatum* type 1 and type 3 posess 2 rRNA operons, which differ by a one base pair insertion that renders sequence analysis difficult. The colonies are dysgonic and colorless (**Fig. 8**).

Table 9

clarithromycin 1000 mg or azithromycin 250 mg
plus
rifampicin 600 mg or 300 mg of rifabutin
plus
ethambutol 15 mg/kg

Table 10

clarithromycin 1000 mg or azithromycin 500 mg
plus
rifampicin 600 mg or 300 mg of rifabutin
plus
ethambutol 25 mg/kg

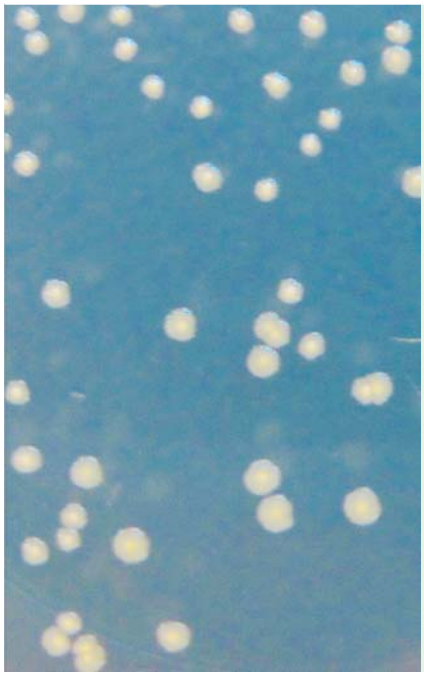


Fig. 8 *M. celatum* on Middlebrook 7H10 agar. [Courtesy of Dr. Elvira Richter, Research Center Borstel, German National Reference Center for Mycobacteria].

Therapy: Drug susceptibility testing reveals probable resistance to INH and RMP, but sensitivity to rifabutin (RBT), EMB, the aminoglycosides and PTH may be variable depending on the strain [201]. Quinolones always seem to be sensitive. Likewise, drug susceptibility testing is recommended here.

Since *M. celatum* rarely leads to pulmonary infection in immunocompetent patients, there is little experience with therapy. A Korean study described that a combination therapy including clarithromycin, EMB and ciprofloxacin was effective, followed by the resection of a persisting cavern [205].

8.1.3 *M. genavense*

Microbiology: *M. genavense* is characterized by extremely poor growth, which in some cases can be so low in liquid cultures that it is not indicated by detection systems. Furthermore, growth is only minimal on agar media and no growth can be achieved on egg based media. If the presence of *M. genavense* in liquid culture without indication of growth is suspected, a smear should be made from the liquid culture that might show the

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typical image of *M. genavense* with very short acid-fast mycobacteria. Also, nucleic amplification techniques (NAT) may be indicated in cases of suspected disease. *M. genavense* is mainly found in HIV-infected patients [206], but there are also single cases known in HIV-negative individuals [207].

Therapy: The optimal treatment is not known, however, it is recommended that clarithromycin is combined with RMP, one FQ, EMB and/or SM/amikacin [1,208,209].

8.1.4 *M. gordonae*

Microbiology: *M. gordonae* is typically detected in tap water [8]. Therefore, the detection of *M. gordonae* from sputum or gastric aspirates or bronchoscopy [104] is usually without clinical significance, given the high probability of contamination with bacteria from tap water. *M. gordonae* preferentially grows at 31 °C on agar plates in moist shiny yellow-orange colonies (**Fig. 9**).

Therapy: There is no mandatory recommendation for therapy regarding *M. gordonae* since the organism is almost always regarded as non-pathogenic. According to ATS/IDSA there is *in vitro* susceptibility to EMB, RBT, clarithromycin, FQ and Linezolid [1].



Fig. 9 *M. gordonae* on Löwenstein-Jensen medium. [Courtesy of Dr. Elvira Richter].

8.1.5 *M. haemophilum*

Microbiology: *M. haemophilum* may be isolated from superficial skin lesions, bronchopulmonary specimens or blood in immunosuppressed patients [91]. Proof is difficult because the bacteria only grow at 31 °C. Growth is very slow and furthermore requires iron supplemention of the medium.

A reliable drug susceptibility testing is not possible due to limited growth. ATS/IDSA outlines *in vitro* sensitivity to RMP, rifabutin, clarithromycin, FQ, and amikacin [1]. Lindeboom et al. recommend a combination of clarithromycin, ciprofloxacin, and an antibiotic from the group of rifamycins [91].

8.1.6 *M. kansasii*

Microbiology: Pulmonary infection with *M. kansasii* is similar to *M. tuberculosis* in its clinical presentation. In rare cases *M. kansasii* is also an etiologic agent of childhood lymphadenitis.

M. kansasii strains showed genetic variability. Six subspecies are distinguished. The strains pathogenic to human mainly belong to subspecies I. *M. kansasii* is one of the photochromogenic species. It can grow at 31 °C and 37 °C in dry and large colonies. Exposed to light a typical yellow pigmentation appears.

It should be noted that the IGRA tests may give a positive result as for *M. szulgai, M. marinum* and *M. flavescens* and thus can't be used for discrimination to tuberculosis [91,92].

Therapy: Diseases caused *by M. kansasii* can usually be treated with a triple combination of INH, RMP and EMB. In most strains the MIC of isoniazid is 1.0 micrograms/ml, and thus in the range of easily accessible serum and tissue levels when treated in normal doses (3 – 5 mg/kg body weight) [210]. Alternatively, treatment with clarithromycin, EMB and RMP is recommended, since all antibiotics have low MIC values. This triple therapy regime, although used intermittently, was efficient [211]. The duration of therapy should be sufficiently long, i.e. include twelve months from sputum conversion [1]. In case of resistance to RMP other drugs like clarithromycin/azithromycin, moxifloxacin, EMB, trimethoprim/sulfamethoxazole or SM should be considered based on the results of drug susceptibility testing [1].

8.1.7 *M. malmoense*

Microbiology: *M. malmoense* grows preferentially at lower temperatures (31 °C), especially on solid media. *M. malmoense* can also cause pulmonary infections, but since these specimens are not routinely incubated at 31 °C, the detection of pulmonary *M. malmoense* infection may be difficult or delayed [212].

Therapy: *M. malmoense* is resistant to INH and RMP, however susceptible to EMB, rifabutin, clarithromycin and moxifloxacin [1] (and own results [ER]). The optimum treatment is not known. The combination of INH, RMP and EMB, with or without clarithromycin/azithromycin and FQ, is reported effective [1]. Adapted to the results of drug susceptibility testing an alternative therapy with clarithromycin, EMB and RMP/RBT is recommended [16,212 – 214].

8.1.8 *M. marinum*

Microbiology: *M. marinum* is the etiologic agent of the swimming pool granuloma and exclusively isolated from superficial lesions and affected lymph nodes [215]. Typically, patients have contact with aquaria. Infections with this mycobacterium are also described as complication of therapies with biological [80,81]. Also, *M. marinum* prefers a growth temperature of 31 °C. The photochromogeneity is a characteristic feature. It should be noted that the IGRA tests, as with *M. kansasii, M. flavescens* and *M. szulgai*, can turn positive, so not suitable for the exclusion of tuberculosis [93,94].

Therapy: *M. marinum* is resistant to INH but susceptible to EMB, RMP and clarithromycin. For treatment of infections with *M. marinum* the use of the two drugs clarithromycin and RMP is recommended for several (four or more) months – depending on depth of wound and progress of healing [216]. The therapy can probably be optimized by the addition of EMB due to the synergistic effect of with RMP [128,217]. Also, the combinations of clarithromycin and EMB as well as RMP and EMB are described as effective [1]. Furthermore, minocycline, doxycycline and trimethoprim/sulfmethoxazol can be used [1]. For in-depth skin and soft tissue infections caused by *M. marinum* a surgical procedure (debridement) can be indicated, but only in combination with drug treatment.

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8.1.9 *M. simiae*

Microbiology: *M. simiae* is a rarely isolated species of mycobacteria, which also is present in the environment [1]. In rare cases, *M. simiae* is responsible for pulmonary infections. Extra-pulmonary localizations are very rare and are mainly described in immunocompromised patients [218].

Therapy: *M. simiae* is resistant to almost all antibiotics [210]. It can be susceptible to clarithromycin, moxifloxacin, and PTH. An optimal combination and duration of therapy has not yet been established. A treatment with clarithromycin, moxifloxacin and trimethoprim/sulfamethoxazole is recommended herein [1].

8.1.10 *M. szulgai*

Microbiology: *M. szulgai* is one of the scotochromogenic mycobacteria with a yellow-orange pigmentation. *M. szulgai* can cause tuberculosis-like disease patterns both in terms of the local findings and general symptoms [219].

It should be noted that the IGRA tests may turn positive, as with *M. marinum, M. kansasii* and *M. flavescens*, so are not suitable for the exclusion of tuberculosis [93,94].

Therapy: In drug susceptibility testing *M. szulgai* is generally resistant to INH and susceptible to EMB, RMP and clarithromycin. *M. szulgai* can be treated with most anti-mycobacterial drugs. The optimal composition and duration of treatment has not been established. Based upon the *M. kansasii* modality (12 months INH, RMP and EMB), the combination using the three drugs RMP, EMB and clarithromycin according to susceptibility testing is probably adequate, but must always be clinically supervised [1].

8.1.11 *M. ulcerans*

Microbiology: On the genetic basis *M. ulcerans* is closely related to *M. marinum* and can only be distinguished by specific genetic analyzes. In contrast to *M. marinum*, growth of *M. ulcerans* is sparse or non-existent on the usual media. It is isolated from the skin lesions of patients with Buruli ulcer [74].

Therapy: Drug therapy of large ulcers is unsatisfactory. Drug susceptibility testing is not possible due to limited growth. For in-depth skin and soft tissue infections, a surgical procedure (debridement) can be indicated, but only in combination with a drug treatment (clarithromycin and RMP or RMP and streptomycin, both recommended for eight weeks) [1]. Thermal therapy for local treatment is also described as a further new therapeutic approach [139].

8.1.12 *M. xenopi*

Microbiology: *M. xenopi* can be detected in tap water and thus, clinical significance in most cases is low. Though it can cause pulmonary infections in patients with other underlying diseases [220].

Therapy: In drug susceptibility testing *M. xenopi* is susceptible to a variety of drugs (RMP, clarithromycin, fluoroquinolones, PTH; variable with INH and EMB), but there is no established effective therapy [220]. For diseases caused by *M. xenopi* ATS/IDSA recommends the same triple combination as for *M. avium* [1]. Isoniazid in-vivo is considered effective, similar to the situation in *M. kansasii* [210].

8.2 Rapidly growing mycobacteria

Rapidly growing mycobacteria are highly resistant to a wide variety of environmental conditions and therefore widely distributed in the environment [8,103]. The detection of rapidly growing mycobacteria from non-sterile clinical specimens can often be caused by contamination. The eponymous feature of rapidly growing mycobacteria (i.e. significant growth in less than seven days at their optimum growth temperature) only applies to standard culture conditions. Cultural growth of rapidly growing mycobacteria in the specimen material can therefore take a longer time.

In routine bacteriology one should always think of rapidly growing mycobacteria and perform an acid-fast staining if small grampositive bacteria of different morphology are present. Rapidly growing mycobacteria usually grow better at lower temperatures (31 °C) compared to the usual temperature used for detection of TB bacteria at 37 °C. Therefore, samples with suspected rapidly growing mycobacteria should be incubated in parallel at lower temperature.

Due to their enormous genetic variability in recent years more and more new, distinct species within the spectrum of rapidly growing mycobacteria have been described. Very few have clinical significance – mostly *M. abscessus* and *M. chelonae*.

8.2.1 *M. abscessus*

Microbiology: The taxonomy of *M. abscessus* has been amended several times in recent years, based on genetic data. The spin-offs of *M. massiliense* and *M. bolletii* from *M. abscessus* [221,222] have now been reversed, resulting in the species *M. abscessus* with two subspecies: *ssp. abscessus* and *ssp. bolletii* [223]. The second includes the former species *M. massiliense* and *M. bolletii*. In patients with cystic fibrosis (CF) *M. abscessus* is one of the most frequently isolated mycobacterial species [224]. After lung transplantation these patients have an increased risk of infection with *M. abscessus* [225]. *M. abscessus* can also cause pulmonary infections or skin lesions in patients without cystic fibrosis [1].

Therapy: Treating diseases caused by *M. abscessus* is difficult. A combination therapy of oral clarithromycin and parenteral administration of amikacin, plus cefoxitin, or imipenem, for a period of 2–4 months, followed by 6–12 months with oral drugs tested as sensitive, may cause improvement, but often there is no definitive cure [1]. Among the macrolides, azithromycin has the possibility of better efficacy than clarithromycin [226]. Moxifloxacin is also effective, but in combination with macrolides an antagonistic effect was observed [227]. In a Taiwanese study the following *in vitro* susceptibility patterns were found for forty isolates: amikacin (95.0%), cefoxitin (32.5%), ciprofloxacin (10.0%), clarithromycin (92.5%), doxycycline (7.5%), imipenem (12.5%), moxifloxacin (22.5%), sulfamethoxazole (7.5%) and tigecycline (100%) [228]. The authors reported a combination therapy of clarithromycin, amikacin and another antibiotic failing in 27.3% twelve months after initiation. Because drug therapy often is not enough an additional surgical procedure may be useful [229]. In pulmonary infections, the duration of therapy after the first negative culture is 12 months [230].

In diseases caused by certain strains of the *M. abscessus subspecies ssp. bolletii* (for a while referred to as *M. massiliense*) the response to therapy with macrolides is better because less often resistance develops [226].

8.2.2 *M. chelonae*

M. chelonae can cause pulmonary infections and skin lesions [1].

Therapy: For diseases caused by *M. chelonae* a therapy with clarithromycin in combination with an antibiotic that is effective *in vitro* is recommended over the course of twelve months after sputum conversion. Usually the pathogen is susceptible to tobra-

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mycin, linezolid and clarithromycin; and in the majority (75%) also to amikacin and moxifloxacin [1]. In pulmonary infections, the duration of therapy is for twelve months after the first negative culture [230].

8.2.3 *M. fortuitum*

Microbiology: The species of the *M. fortuitum* group (*M. fortuitum, M. peregrinum, M. mucogenicum, M. senegalense, M. septicum* and an unnamed group [biovar [3]]) are largely without clinical significance. Infections may occur in exceptional cases, e.g. traumatic wound infections or surgery.

Therapy: *M. fortuitum* is almost always susceptible *in vitro* to amikacin, ciprofloxacin, levofloxacin, moxifloxacin, imipenem and sulphonamides [1]. Approximately 80% of the pathogens also are susceptible to cefoxitin, clarithromycin and linezolid. The parenteral administration of amikacin and cefoxitin for 2–6 weeks, followed by oral administration of TMP/SMX and fluoroquinolone for 2–6 months is recommended. In pulmonary infections, the duration of therapy after the first negative culture is twelve months [230].

9 Appendix 2: Properties of the drugs used in the treatment of NTM disease (in alphabetical order)

The following table includes essential information on antituberculous drugs, and is based on the DZK and DGP treatment recommendations for tuberculosis (see [135] for further details). The table also includes agents that are used specifically in the treatment of non-tuberculous mycobacteriosis.

Amikacin (AK)

Amikacin is a synthetic derivative of kanamycin. The mechanism of action, pharmacokinetics and the bactericidal potency are similar to that of streptomycin. The daily dose is 15 mg/kg body weight (once daily, maximum dose 1000 mg). Adverse effects occur as with streptomycin, but renal toxicity is more pronounced.

Azithromycin

Oral once daily dose is 250–500 mg. Absorption is approximately 40% after oral administration of 500 mg. Azithromycin is metabolised predominantly through hepatic pathways.

Common side effects include diarrhoea and nausea. In long-term use there appears to be a significantly increased risk for serious and sometimes fatal arrhythmias.

Contraindications include besides allergy in particular the coadministration of statins and relevant cardiac rhythm disturbances. Macrolides are potent inducers of cytochrome P450-enzymes and therefore lead to a variety of drug-drug interactions (see package insert).

Clarithromycin

The oral daily dosage is 500 mg BD. Absorption after oral administration is approximately 50%. The maximum serum concentration after an oral dose of 500 mg is 3–4 µg/ml. Clarithromycin undergoes hepatic metabolism and is mainly excreted via the kidneys.

Common side effects include nausea, taste disturbances and diarrhoea. Rare but serious ADRs include hearing loss (audiometry is necessary in long term use) and the induction of cardiac arrhythmias (QT prolongation, torsade de pointes).

Contraindications include besides allergy in particular the coadministration of statins and relevant cardiac rhythm disturbances. Macrolides are potent inducers of cytochrome P450-enzymes and therefore lead to a variety of drug-drug interactions (see package insert).

Chinolones

The WHO advises against the use of ciprofloxacin in the treatment of tuberculosis. Levofloxacin and moxifloxacin are the recommended quinolones for use in NTM diseases.

Ethambutol (EMB)

The oral once daily dose is 15 mg/kg body weight (maximum dose 2000 mg). At a dose of 25 mg/kg the drug attains a peak serum concentration of 5 mg/l. EMB is well absorbed (80–85%) from the gastrointestinal tract. There is relatively good diffusion into tissues and body fluids. The blood-brain barrier is crossed only in the presence of inflamed meninges. EMB does enter the fetal circulation, although concentrations in breast milk are unknown.

The drug undergoes predominantly renal elimination, and requires dose adjustment in renal failure. Absorption is reduced by coadministration of antacids resulting in decreased serum concentrations.

Adverse drug reactions: The main adverse drug effect is retrobulbar optic neuropathy resulting initially in the loss of red-green-discrimination and subsequently irreversible ocular damage (visual loss, central scotoma, photophobia). EMB is therefore contraindicated in patients who have impaired vision, those who cannot articulate visual changes (e.g. very young or comatose patients), and in patients with serious underlying eye disease.

Ocular adverse drug reactions occur in approximately 2.6% of patients treated with a daily dose of 25 mg/kg and less than 1% of patients treated with a daily dose of 15 mg/kg. Baseline and follow-up ophthalmological examinations (e.g. once a month) are therefore mandatory. Colour vision should be tested (e.g. Ishihara panel) prior to treatment initiation and regular follow-up examinations are recommended. EMB-associated retrobulbar neuropathy may persist for months, but is usually reversible.

Rare: arthralgia.

Very rare: Cutaneous ADRs and polyneuropathy.

Contraindications: Allergy to EMB, severe pre-existing eye problems (e.g. damage of the opticus nerve, severe diabetic retinopathy, etc.), inability to report visual disturbances (e.g. in advanced age and small children), and severe renal impairment.

Isoniazid (INH)

Dosage is 5 mg/kg body weight (maximum daily dose 300 mg). The drug is almost completely absorbed from the gastrointestinal tract. It rapidly diffuses in tissues and body fluids, and crosses the blood-brain barrier. INH enters fetal circulation and breast milk (see pregnancy and lactation). Parenteral (i.v. and i.m.) administration is possible. The majority of hepatic metabolism (80%) is by means of enzyme acetyltransferase. Metabolism is altered in genetically determined slow and rapid acetylators, but this has limited clinical significance. The peak serum level after 300 mg is 6.5 mg/l for rapid acetylators and 10 mg/l for slow acetylators. After hydrolyzation and conjugation the metabolites are for the most part eliminated via the kidneys.

Tolerability is good.

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Important interactions include: Increased serum levels by prednisolone, paraaminosalicylic acid, and protionamide. INH increases the serum levels of coumarins, phenytoin, valproate, theophylline, carbamazepine and diazepam, and leads to reduced serum levels of azoles.
The interaction of INH and pyridoxine (vitamin B6) metabolism may be a factor in the development of peripheral neuropathy. Administration of pyridoxine 50 mg daily is recommended for patients with increased risk for peripheral neuropathy, in pregnancy, pernicious anaemia and established polyneuropathy.
Important adverse drug reactions: Common: Hepatotoxicity, cutaneous (acne) adverse drug reactions, impaired concentration.
Rare: Hepatitis, neuropathy, lowered threshold for convulsive seizures, and skin rash.
Very rare: Aplastic and hemolytic anemia, agranulocytosis, drug-induced lupus erythematosus, seizures, vertigo, loss of consciousness, optic neuritis, arthralgia, gynaecomastia, and alcohol intolerance (pathological intoxication).
Absolute contraindications include: Isoniazid allergy, acute hepatitis, severe disorders of haemostasis and haematopoiesis.
Relative contraindications include: Seizures, psychosis, clinically significant peripheral neuropathy.

Levofloxacin

Oral daily dose is 15 mg/kg body weight; maximum dose 1000 mg/day).
Very good oral absorption, leading to high tissue concentrations. Hepatic metabolism is minimal, since about 90 % of unchanged drug is excreted renally.
Levofloxacin forms complexes with iron, calcium, magnesium, zinc, vitamins, and sucralfate, which strongly reduce oral absorption. Concomitant administration of these drugs and milk products must therefore be avoided. There is also an important interaction with didanosine leading to reduced serum concentrations.
Adverse effects include CNS ADRs (headache, insomnia and bad dreams), gastrointestinal ADRs (nausea, vomiting), tendon rupture (avoid concomitant use of steroids), liver toxicity, heart rhythm disturbances and photosensitivity.
Contraindications include pregnancy, allergy and QT prolongation. Patients < 18 years old must be advised of the risk of cartilage injury.

Linezolid

Linezolid can be used in the treatment of NTM strains with complex drug resistance *in vitro*. The daily dose should not exceed 600 mg, since significantly more ADRs occur at higher doses (e. g. 600 mg BD). There is excellent oral bioavailability. Linezolid is metabolised by oxidation in the liver and eliminated by urinary excretion.
Unless there is no alternative, linezolid should not be taken for longer than four weeks. The drug is extremely expensive and commonly leads to adverse effects with long-term use (including liver dysfunction, prolonged thrombocytopenia, optic neuropathy with blindness). Patients should be educated about these risks.
Since linezolid inhibits monoamine oxidases, there is considerable potential for drug-drug interactions (see package insert).

Moxifloxacin

Oral daily dosage is 400 mg. Moxifloxacin has a rapid bactericidal effect on proliferating intra-and-extracellular pathogens. The drug is eliminated unchanged both hepatically and renally. An important interaction is the formation of complex enteral bonds with iron, calcium, magnesium, zinc, vitamins and sucralfate, which reduce the absorption greatly.
Concomitant administration of these drugs and milk products must therefore be avoided. Adverse effects include CNS ADRs (headache, insomnia and bad dreams), gastrointestinal ADRs (nausea and vomiting), tendon rupture (avoid co-administration with steroids), arrhythmias, liver toxicity and photosensitivity.
Contraindications include pregnancy, fluoroquinolone allergy and QT interval prolongation. Patients < 18 years old must be advised of the risk of cartilage injury.

Protionamide

Daily dosage is 15 mg/kg body weight (maximum dose 1000 mg) if no INH is given. With concomitant INH administration, dosage is reduced to 7.5 mg/kg body weight (maximum daily dose 500 mg). After oral administration absorption is approximately 70 %. There is good tissue and cerebrospinal fluid penetration. The metabolism is exclusively hepatic (95 %), and metabolites are excreted in urine. The main undesirable effect is pronounced gastrointestinal intolerance, which can be reduced in some cases by switching to a twice-daily regimen (2 × 500 mg).
More ADRs include liver toxicity, functional disorders of the CNS and depression (to the extent of suicidal tendencies), thyroid disorders and allergies.
It is important to screen for diabetes as protionamide can lead to hypoglycaemia.

Rifampicin (RMP)

Dose is 10 mg/kg body weight (maximum daily dose 600 mg). The maximum serum concentration of 600 mg is 7 – 8 mg/l. RMP is almost completely absorbed from the gastrointestinal tract and rapidly diffuses (with moderate penetration of the blood-brain barrier) into tissues and body fluids, and enters the foetal circulation and breast milk. Parenteral administration is possible. RMP initially causes a rapid induction of degrading enzymes. Up to 95 % of RMP is metabolised in the liver and eliminated in bile.
Tolerability is good.
RMP induces hepatic microsomal enzymes, particularly the cytochrome P-450 complex, leading to many potential drug-drug interactions. The most important interaction is with systemically-administered hormonal contraceptives, leading to reduced efficacy of both drugs. RMP causes reductions in the serum levels of coumarins, glucocorticoids, tamoxifen, L-thyroxine, sulfonylureas, diazepam, zolpidem, methadone, digoxin, digitoxin, verapamil, nifedipine, beta-blockers, ACE inhibitors, sartans statins, theophylline, cyclosporine, azoles, clarithromycin, doxycycline, atovaquone and chloramphenicol. Important interactions exist with protease inhibitors and non-nucleoside reverse-transcriptase inhibitors (see the chapter on patients with HIV infection).
Important adverse drug reactions: Always: red coloration of body secretions (urine, faeces, tears). Caution: Soft contact lenses change colour.
Common: Mild hepatotoxicity (transaminases < 3 × ULN), cholestasis.
Rare: Hepatitis, cutaneous drug reactions, gastrointestinal intolerance and mild haematological abnormalities (thrombocytopenia), flu-like syndrome (with intermittent administration, im-

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munological activation with antibody formation, the symptoms range from flu-like syndrome to severe immunological disorders).
Very rare: Acute renal failure and haemolytic anaemia (RMP should be discontinued and not re-challenged), anaphylaxis, CNS ADRs (fatigue, headache, dizziness, vertigo, ataxia, confusion, blurred vision, fatigue).
Immunological hypersensitivity reactions to RMP: Therapy with RMP should be discontinued immediately and should not be re-challenged in patients who develop a "flu-like syndrome," thrombocytopenia, haemolysis, or incipient renal failure.
The following are considered absolute contraindications: allergy to RMP, acute hepatitis, bile duct obstruction, and severe liver impairment (Child Pugh C).

Rifabutin (RFB)

The mechanism of action is similar to that of rifampicin. The recommended once daily dosage is 300 mg. The dose should be reduced to 150 mg every second day if co-administered with protease inhibitors.
Absorption after oral administration is poor (10 – 20 %), but this is compensated for by excellent tissue penetration leading to therapeutic tissue concentrations. RFB is excreted via bile and urine. Drug-drug interactions and adverse drug effects are comparable to that of rifampicin. High doses (450 mg daily) often lead to uveitis (eye pain, visual impairment).

Streptomycin (SM)

The recommended once daily dose is 15 mg/kg body weight (maximum dose 1000 mg). A dose of 1 g leads to peak serum concentrations of 25 – 45 mg/L. SM is only available for parenteral administration (intramuscularly or intravenously). It diffuses moderately well into tissue and body fluids, and penetrates the blood-brain barrier only in inflamed meninges. SM enters fetal circulation and breast milk. Most of the drug undergoes renal elimination in unchanged form. For this reason dose adjustment is required in renal impairment.
Tolerability is relatively good.
The most important shared drug toxicities relate to ototoxicity and nephrotoxicity of aminoglycosides. Coadministration of other potentially ototoxic or nephrotoxic drugs (e.g. cephalosporins, amphotericin B, colistin, cyclosporine, cisplatin, loop diuretics, methoxyflurane, tenofovir and macrolides) should be avoided, if possible.
Adverse drug reactions: The main adverse drug reactions include damage of the eighth cranial nerve (preferentially affecting vestibular function) and diffuse tubular injury leading to renal impairment. Therefore, baseline audiometry and measures of renal function with regular monitoring during therapy (every 4 weeks for audiometry and weekly for renal function) is recommended. It may also be necessary to monitor serum trough levels to avoid toxicity in high-risk patients.
The risk of ototoxicity is dose-dependent: with a daily dose of 2 g the risk is 60 – 120 % after 75 days of therapy; but reducing the dose to 1 g daily the risk drops to 30 % after 120 days on therapy. In addition to dose, risk increases with age, the extent of pre-existing renal impairment or inner ear damage, as well as administration of other ototoxic drugs. The ototoxicity is usually reversible, although severe vestibular damage can be long lasting.
Rare: Tinnitus, cutaneous ADRs.
Very rare: Impaired renal function, agranulocytosis, aplastic anaemia, anaphylaxis, neuromuscular blockade, respiratory depression, paraesthesia, exfoliative dermatitis, hypersensitivity reactions with fever, rashes and eosinophilia. Contact allergies are described for e.g. nursing staff.
Contraindications include: Allergy to SM, pregnancy, severe renal impairment, cochlear or vestibular damage and uncontrolled or severe myasthenia.

Other agents

Rarely used antibiotics in the treatment of NTM disease include trimethoprime/sulmethoxazole (TMX/SMZ), doxycycline and imipenem. Refer to package inserts for details on the properties of these agents. Cefoxitin is not available in Germany (refer to package insert).
Dosage (NTM disease):

- Trimethoprim/Sulfamethoxazole: Oral 2 × 160/800 mg/day
- Cefoxitin: 12 g/day either 4 × 3 g i. v. or 6 × 2 g i. v.
- Imipenem: 4 × 1 g i. v.
- Doxycycline: orally 100 mg/day

Glossary

ATS	ATS American Thoracic Society
BAL	Bronchoalveolar lavage
COPD	Chronic Obstructive Pulmonary Disease
CF	Cystic Fibrosis
EMB	Ethambutol
FQ	Fluoroquinolone
GERD	Gastroesophageal reflux disease
HIV	Human Immunodeficiency Virus
HRCT	High Resolution CT
IDSA	Infectious Diseases Society of America
IGRA	Interferon-γ release assay
IFN-γ	Interferon-γ
IL	Interleukin
INH	Isoniazid
IRIS	Immune Reconstitution Inflammatory Syndrome
MAC	*M. avium-complex*
MOTT	Mycobacteria other than tuberculosis (mycobacteria not belonging to the *M. tuberculosis-complex*)
MRI	Magnetic Resonance Imaging
NAT	Nucleic Acid Amplification Test
NTM	Non-tuberculous Mycobacteria
rDNA	Ribosomal RNA encoding DNA
RBT	Rifabutin
RMP	Rifampicin
SM	Streptomycin

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Competing interests

None.

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