Identification and Drug Treatment of Nontuberculous Mycobacteria Isolated from Patients Lived in Greece during 2000 – 2017

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Introduction

The most well-known mycobacteria in medicine are those that cause tuberculosis and leprosy (e.g. M. Tuberculosis). Moreover, these mycobacteria are not detected in the environment and are transmitted from human to human. But, another group of mycobacteria called “nontuberculous mycobacteria” (NTM) that have been found to be widely dispersed in the environment are considered potentially pathogenic for humans and animals. Although the NTM cause diseases in humans and animals (e.g. M. avium - intracellulare causes disease in poultry and pigs), human to human or animal to human transmission is very rare. These NTM have been isolated from numerous environmental sources, such as surface water, drinking (tap) water and soil [1-12].

More than 169 NTM species have been identified until today. These NTM have been determined from hospitalized patients and environmental samples. They are potential pathogens, principally to immunosuppressed and immunocompetent patients (e.g. transplant recipients, AIDS and cancer patients), and to vulnerable groups such as children, the chronically ill and the elderly. Generally, populations at risk include individuals who suffer from lung disease and a weakened immune system [1,5,13-23].

Since there is no statutory framework for the recording of atypical mycobacteria isolated from patients, we do not get an overall view of their effect on human health.

Abstract

Background: Until today, the epidemiology of Non-Tuberculous Mycobacteria (NTM) is unknown because NTM disease is not notifiable in most countries worldwide. Chronic pulmonary infection is the most common clinical manifestation. Host and pathogen factors leading to NTM disease are not well understood and preventive therapies are lacking. These organisms have been mainly isolated from water, soil and dust, which are proposed to be the ways through NTM infect human and animal.

Methods: Research studies published during 2006-2017 concerning isolation and identification of NTM in Greece for the period 2000-2017 are taken into consideration. NTM status in Greece was compared with relative recent studies worldwide.

Results: In Crete, the most frequently isolated species was M. gordonae (30.3%), followed by M. fortuitum (25.0%) during 2000-2004. When another research work in Crete for the period 2000-2009 was taken into consideration, M. gordonae again was the most frequently isolated NTM species (16.9%) but now M. lentiflavum (14.1%) has become the second most frequently isolated species. In Thessaly, M. gordonae was also predominant (31.0%), followed by M. fortuitum (35.6%) during 2004-2006. The study in Athens, for the period 2007-2013, showed that M. lentiflavum (33.6%) was now predominant, followed by M. gordonae (13.9%). Lastly, in Larissa, M. fortuitum was predominant (30.8%), and M. gordonae (22.7%) was still dominant among isolated NTM species that were isolated during 2003-2013. Worthy to notice that 4.9% of the isolated NTM in Athens study was not identified while the corresponding percentage in Larissa study was 23.0%. Only one systematic study concerning drug treatment of NTM isolated in Greece was carried out during 2000-2017.

Conclusion: The prevalence of NTM is underestimated because diagnostic procedures are not optimized specifically for NTM, as well as systematic studies are few and there is not a national reference laboratory to record all NTM isolations in Greece.

Keywords: Non-tuberculous mycobacteria, Epidemiology, Pulmonary disease, Nontuberculous mycobacteria antibiotic susceptibility, Greece
Only sporadic studies are published, mainly showing that NTM cause lymphadenitis and infections of the lungs, skin, soft tissue, bursa, joints, tendon sheath, and bones. These studies show that NTM diseases occur mostly in the developed world, and the data suggest transmission to humans by means of water. The latest data in literature show the increased incidence is not localized geographically. Thus, it may be attributed to the interest obtained by the researchers, due to AIDS patients with NTM infections. It is estimated that 25-50% of patients with AIDS have been infected by NTM [6,7,13,16,17,24-33].

As NTM are not notifiable in most European countries and in Greece, the epidemiological situation of more than 169 NTM species is largely unknown [34].

The aim of this study was to present available data from specific areas of Greece on NTM. The methods NTM needed in order to be isolated and identified, and the time period each research work being performed, as well as the kind of the studied biological samples and drug treatment is included. Additionally, these data are compared with NTM data published in other countries.

**Epidemiology of NTM**

Both the American Thoracic Society (AST) and the British Thoracic Society (BTS) have issued guidelines for the diagnosis of NTM disease.

The main clinical forms of the NTM disease are pulmonary disease, lymphadenitis, skin and soft tissue disease and generalized disease [13,35,36].

The predisposing factors for NTM disease are shown below:

- Age (40-70 years old usually)
- Environment (beaches)
- Agricultural areas
- Silicosis, pneumoconiosis
- Chronic obstructive pulmonary disease
- Bronchiectasis
- Radiological lesions of previous tuberculosis
- HIV disease
- Malignancy
- Diabetes
- Alcohol Abuse
- Warm climate

Environment (water, soil and dust) is considered the main source of human NTM infections. Until today, *M. avium complex* is the best studied of them. Environmental studies in the USA have shown that *M. avium complex* is developed in natural water springs, especially in the southeastern regions. The air transport of these organisms is likely to be an additional mechanism of transmission of the disease but it is generally accepted that the natural water sources and drinking water are the main source of human infection for the *M. avium* complex. Chronic pulmonary disease is the most common clinical manifestation of disease caused by atypical mycobacteria. *M. avium complex* (MAC), followed by *M. kansasii*, is the most common pathogen causing lung disease in the United States. Other pathogens that occasionally cause pulmonary disease are *M. abscessus*, *M. fortuitum*, *M. szulgai*, *M. malmoense* and *M. celatum*. *M. xenopi* is the second post-*M. avium complex* cause of atypical pulmonary disease in areas of Canada, the United Kingdom and other regions of Europe, while *M. malmoense* is second to *M. avium complex* in Scandinavia and other regions of Northern Europe. The *M. avium* affects people with underlying respiratory diseases such as chronic obstructive pulmonary disease (COPD), or immunocompromised patients, such as patients with lung cancer or extrapulmonary cancer.

Children rarely develop disease from NTM, except those with cystic fibrosis. In a study on generalized atypical tuberculosis in AIDS patients, it has been found that some cases are likely to come from hospital drinking water. Additionally, the *M. avium complex* has the ability to be transmitted by the oral route as well. *M. malmoense* has emerged as one of the main pathogens in northern Europe and has also been found in the Finnish waters, the territory of Zaire and Japan, while *M. simiae* in countries such as Israel, Cuba and southwest America [23,26,34,37-49].

The different NTM species are divided between rapid growers (RGM), which develop visible colonies in solid media within 7 days, and slow growers (SGM), which require longer incubation times. Growth rate is clinically important because RGM and SGM differ in their antimicrobial susceptibility and patterns of disease. Specifically, SGM are most often responsible for pulmonary and Lymph node diseases, whereas RGM are more commonly isolated from skin and osteoarticual infections.

The clinical symptoms of atypical tuberculosis are nonspecific and can easily be confused with tuberculosis caused by *M. tuberculosis* complex or underlying pulmonary disease. They include dry or productive cough, mucus sputum, bloody sputum or hemoptysis, weight loss, easy fatigue, night sweats, dyspnea in fatigue or serenity, swelling of submandibular and cervical lymph nodes and therefore must be differentiated from the symptoms of *M. tuberculosis* complex infection or those of the underlying pulmonary disease. The absence of specific diagnostic features in physical examination, chest history and radiography and the reduced diagnostic value of dermal tests make isolation and identification of NTM essential for diagnosis. Thus, the treatment of atypical tuberculosis is an issue on which there is no consensus, because large clinical trials are lacking in order to propose international guidelines [50,51].

**Materials and Methods**

**Materials**

Five research works published between 2006 and 2017 concerning positive NTM patients who lived in various geographical areas of Greece during the period 2000-2017 have been taken into consideration. In these research works, the studied groups and the methods used for the determination and identification of NTM from patients lived in (a) Thessaly area, central Greece, (b) Athens and (c) Crete Island are mainly presented (Figure 1) [13,17,36,38,52].

The earliest of them published in 2006 and used different methods to analyze 76 nontuberculous mycobacterial isolates from Crete, Greece, for the period 2000-2004. The next
systematic study was the work of Gerogianni et al. 2008 which concerned the isolation of NTM in respiratory specimens taken from 564 positive NTM patients lived in Thessaly region, central Greece, for the period 2004-2006. Next, Gitti et al. published in 2011 a research work concerning clinical significance and antibiotic susceptibility of NTM from patients in Crete, Greece, for the period 2000-2009. Study of Panagiotou et al. regarding the epidemiology of pulmonary NTM isolated from patients at General Hospital in Athens, Greece for the period 2007-2013, was published in 2014. Consequently, in 2016, another research working team of Dovriki et al. studied the way that NTM infect humans. In this study, positive NTM patients lived in Larissa city, Thessaly region, central Greece, for the period 2003-2013 were included.

Based on the above research works and also on the case studies published in the period 2006-2017 concerning Greece, information on laboratory routines for detection and identification of NTM and data on the number and type of NTM species identified in pulmonary as well as non-pulmonary specimens and drug treatment have taken into consideration [53-58].

Additionally, corresponding research data from European and global studies are taken into consideration to compare NTM “status” in Greece with them [23,26,34,39-49].

Clinical specimens processing

Methods that were used for digestion and decontamination of clinical samples to recover *M. tuberculosis* have also proved to be useful for the NTM. After sample preparation (liquidation, homogenization, decontamination and neutralization), staining and microscopy took place. Once decontaminated, samples may be grown on ordinary solid media for mycobacteria such as egg-based Lowenstein-Jensen (LJ), agar based medium (Middlebrook) or selective media (LJ Gruft; Difco Laboratories, Oxford, UK). Liquid media cultures include the automated Bactec MGIT 960. Consequently, the identification of mycobacteria to species level was performed using the Genotype CM and MTBC commercial kits (Hain Lifescience, Germany) or Accuprobe commercial kit [17,24,52,54,59].

**Results**

As mentioned above, in the present study the results of the research works concerning the areas of Thessaly, Larissa, Athens and Crete were mainly taken into consideration. Specifically, in the research work of Gitti, et al. 2006, 76 positive NTM patients who lived in Crete over a 5-year period are included, while in the next research work in the same geographic area 207 positive NTM patients are included between 2000 and 2009. The study in Thessaly included 214 positive NTM patients (68.7% male) for the period 2004-2006 while in Larissa area 367 positive NTM patients (68.0% male) are included during 2003-2013. The study of Panagiotou, et al. 2014 included 120 positive NTM patients (63.0% male) who lived in Athens for the period 2007-2013 (Table 1).

The identification of NTM species was carried out in tertiary referral hospitals (Heraldion University Hospital; Crete, Larissa University Hospital; Thessaly and Sismanoglio - A. Fleming General Hospital of Attiki; Athens, respectively) in all the included research works.
As it can be seen from Table 1, in Crete, the most frequently isolated species was *M. gordonae* (30.3%), followed by *M. fortuitum* (25.0%), *M. fortuitum-M. chelonae complex* (15.8%) and *M. avium* (11.8%) during 2000-2004. When the second research work studied patients lived in Crete for the period 2000-2009 was taken into consideration, *M. gordonae* again was the most frequently isolated NTM species (16.9%) but now the one followed was different compared with the previous work; *M. lentiflavum* (14.1%) has become the second most frequently isolated species, followed by *M. fortuitum* (9.0%) and *M. avium* (7.6%). In Thessaly, *M. gordonae* was also predominant (31.0%), followed by *M. fortuitum* (35.6%), *M. peregrinum* (23.8%) and *M. avium* (4.6%) during 2004-2006. The study in Athens, for the period 2007-2013, showed that *M. lentiflavum* (33.6%) was now predominant, followed by *M. gordonae* (13.9%), *M. avium* (13.1%) and *M. fortuitum* (12.3%). Lastly, in Larissa, *M. fortuitum* was now predominant (30.8%), and *M. gordonae* (22.7%) was still dominant among isolated NTM species, followed by *M. peregrinum* (12.0%), *M. chelonae* (9.2%) and *M. avium* (2.1%) that were isolated during 2003-2013. Worthy to notice that 4.9% of the isolated NTM in Athens study was not identified while the corresponding percentage in Larissa study was 23.0%.

Additionally, the specimen types from which NTM were isolated were mainly sputum (90% approximately) and bronchial washings while all the kind of biological samples included in the studies of Crete published in 2011 and Larissa published in 2016. The research works in Crete used the commercial kits Accuprobe and Genotype while the others used only the commercial kit Genotype for identification of NTM species.

Also, there was only one systematic study and few case studies of NTM isolated from patients living in Greece, for the years 2000-2009, 2010, 2011 and 2017 that referred to drug treatment [17,53-58].

The study of Gitti et al. 2011 was the only systematic work in Greece concerning the study of drug treatment of positive NTM patients in Crete. It covers the period 2000-2009 and the drug susceptibility / resistance per studied NTM is presented in Table 2. Additionally, few published case studies referred to drug treatment of specific NTM species isolated from Greek patients. In Korres, et al 2006 case study the M. chelonae drug susceptibility is presented; *M. lentiflavum* and its resistance status is presented in the case study of Neonakis, et al. 2007; Neonakis, et al. 2010 case study the biochemical profile of M. arupense and its resistance status are presented; Bamias, et al. 2011 case study present an atypical mycobacteria skin infection and the drug treatment used; the drug treatment of *M. kansasii* is presented in the case study of Mazis, et al. 2011; and lastly *M. avium complex* drug treatment is presented in Gerogianni et al. 2007 case study (Table 2).
Table 2: Antimicrobial agents used against the NTM species isolated from patients in Greece and their drug susceptibility / resistance.

<table>
<thead>
<tr>
<th>Paper</th>
<th>NTM species</th>
<th>Drug treatment</th>
<th>Susceptibility / Resistance</th>
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<tbody>
<tr>
<td>Gitti et al. 2011</td>
<td>Slowly growing NTM (SGM)</td>
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<td></td>
<td>M. avium</td>
<td>Aminoglycosides (Streptomycin, Amikacin, Tobramycin)</td>
<td>M. avium &amp; M. intracellulare (Clarithromycin, Azithromycin / Aminoglycosides, Quinolones, Others)</td>
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<td></td>
<td>M. intracellulare</td>
<td>Macrolides Clarithromycin, Azithromycin</td>
<td>M. KSansii (Streptomycin, Amikacin, Macrolides, Quinolones, Isoniazid, Rifampicin, Ethambutol, Ethionamide, Linezolid, TMP-SMX / Tigecycline)</td>
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<td></td>
<td>M. kansasii</td>
<td>Quinolones (Ciprofloxacin, Levofloxacin, Moxifloxacin)</td>
<td>M. scrofulaceum (Amikacin, Macrolides, Rifampicin, Ethambutol, Ethionamide, Linezolid, TMP-SMX / Streptomycin, Quinolones, Isoniazid, Tigecycline)</td>
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<td></td>
<td>M. marinum</td>
<td>Others (Isoniazid, Rifampicin, Ethambutol, Ethionamide, Linezolid, Tigecycline, Trimethoprim-Sulfamethoxazole (TMP-SMX), Cefoxitin, Imipenem, Amoxicillin-Clavulanic acid (AMC), Doxycycline)</td>
<td>M. marinum (Aminoglycosides, Macrolides, Quinolones, Ethambutol, Ethionamide, Linezolid, Trimethoprim-Sulfamethoxazole / Isoniazid, Rifampicin, Tigecycline)</td>
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<td></td>
<td>M. gordonae</td>
<td></td>
<td>M. gordonae (Amikacin, Macrolides, Quinolones, Isoniazid, Rifampicin, Ethambutol, Ethionamide, Linezolid, TMP-SMX / Tigecycline)</td>
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<td></td>
<td>M. xenopi</td>
<td></td>
<td>M. xenopi (Aminoglycosides, Macrolides, Quinolones, Rifampicin, Ethambutol, Ethionamide, Linezolid)</td>
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<td></td>
<td>Rapidly growing NTM (RGM)</td>
<td></td>
<td>M. fortuitum (Aminoglycosides, Macrolides, Quinolones, Others)</td>
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<td></td>
<td>M. fortuitum</td>
<td></td>
<td>M. peregrinum (Aminoglycosides, Macrolides, Quinolones, Tigecycline / Doxycycline, Linezolid)</td>
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<td>M. chelonae</td>
<td></td>
<td>M. chelonae (Tobramycin)</td>
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<td></td>
<td>M. abscessus</td>
<td></td>
<td>M. abscessus (Tobramycin, Imipenem / Doxycycline, Linezolid, Cefoxitin, TMP-SMX, AMC)</td>
</tr>
<tr>
<td>Korres et al. 2006</td>
<td>M. chelonae</td>
<td>E-test method (Clarithromycin, imipenem, amikacin, cefoxitin, doxycycline, cotrimoxazole, ciprofloxacin, linezolid)</td>
<td>M. chelonae (Ciprofloxacin, clarithromycin, imipenem with cilastatin)</td>
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<td>(A 55-year-old farmer was admitted to the hospital with low back pain without neurological compromise and nodulopustular skin lesions in the thighs and forearms. The patient reported recurrent episodes of right elbow bursitis as well as right heel pain during the last 8 months).</td>
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<td>Neonakis et al. 2007</td>
<td>M. lentiflavum</td>
<td>Conventional proportions method using LJ slants incorporating the relevant antibiotics (BioMerieux, Marcy l’ Etoile, France and Liofilchem, Roseto, Italy) (Isoniazid, rifampicin, ethambutol, pyrazinamide, streptomycin, para-aminosalicylic acid, capreomycin, pefloxacin, ethionamide, amikacin, nicotinamide, kanamycin, rifabutin, cycloserine, rifampenit, ofloxacin and pyruvate)</td>
<td>M. lentiflavum (Resistance to the majority of the anti-mycobacterial drugs)</td>
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<td>(48 positive NTM cultures over a 3-y period from patients lived in Crete during 2003-2005))</td>
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</table>
Neonakis et al.  
2010 (case study)

M. arupense  
(Pulmonary infection of a 62 year old man from Crete with a malignant mass in his left kidney, who presented with a one-month history of recurrent fever, dyspnea (and haemoptysis)

E-test method  
(Amikacin, ciprofloxacin, clarithromycin, ethambutol, ethionamide, linezolid, levofloxacin, rifampicin, streptomycin, trimethoprim-sulfonmethoxazole)

M. arupense  
(Amikacin, clarithromycin, ethambutol, linezolid, streptomycin / Ciprofloxacin, trimethoprim-sulfonmethoxazole)

Bamias et al.  
2011 (case study)

M. chelonae  
(Skin infection of a 60 year old Caucasian woman with ulcerative colitis with fever and rash involving the right leg who had been treated with corticosteroids and azathioprine)

Cefotixin, clarithromycin, amikacin

M. chelonae  
( Clarithromycin / Infliximab, azathioprine)

Mazis et al.  
2011 (case study)

M. kansasi  
(Clinical tenosynovitis of a 45 year old woman with type I diabetes mellitus, without fever, no weight loss, no symptoms of pulmonary disease)

TREK microbroth dilution method  
(amikacin, ciprofloxacin, clarithromycin, ethambutol, ethionamide, isoniazid, linezolid, moxifloxacin, rifampin, rifabutin, streptomycin)

M. kansasi  
( Clarithromycin, rifampin, rifabutin, isoniazid / ethambutol, amikacin, ciprofloxacin, streptomycin)

Gerogianni et al.  
2017 (case study)

M. avium complex / M. intracellulare  
(Intense backpain of a 68 year old woman with type 1 diabetes mellitus, without fever, no weight loss, no symptoms of pulmonary disease)

Azithromycin, rifampicin, ethambutol, streptomycin

M. avium complex / M. intracellulare  
(Azithromycin, rifampicin, ethambutol, streptomycin)

Discussion

It is well known that NTM are a diverse group of mycobacteria that cause a wide range of human diseases but their pathogenicity varies among the species. Pre-existing lung disease (COPD, cystic fibrosis, idiopathic pulmonary fibrosis, bronchiectasis) smoking, alcoholism, cyanotic heart disease, inherited or acquired immunological disorders and drug-induced immunosuppression with classic chemotherapeutics or newer immunomodulating agents (TNF-α inhibitors) have been associated with the increased incidence of NTM disease recently. Unfortunately, until today NTM detection is not notifiable in most countries worldwide and therefore only some sporadic data is available. Despite the fact that the number of patients with an NTM infection increases rapidly, the respective incidence of clinically significant NTM disease remains unknown due to the lack of systematic reporting [23,26,34,39-49].

As mentioned above, during 2000-2016 only five systematic research studies carried out included positive NTM patients who lived in specific areas of Greece. Also there are only sporadic case studies of NTM isolation and identification. Assuming that the distribution of NTM will be predominately affected by geographical location rather than national borders, the frequency of NTM detection in these studies might well be representative for Greece.

In all the included studies in Greece approximately 94% of the isolates stemmed from respiratory specimens. This is similar to other studies reporting that around 90% of all NTM isolates were respiratory specimens. This is in agreement with other studied in Europe and worldwide [23,34,61,62].

Besides Greece, in most countries NTM are detected by smear microscopy and by both solid and liquid culture. Because only few university hospital laboratories in Greece are performing isolation and identification of NTM, this allows for high quality and standardization of species identification. However, together with the fact that clinical and laboratory diagnosis of NTM is challenging it might result in an underestimation of the true prevalence of NTM because isolates need to be sent from the laboratory that performed the initial diagnostic test to the laboratory that is able to do the NTM species identification. These laboratories use methods optimized for detection of M. tuberculosis complex also for culturing of NTM in general. Both the difficulty in discriminating NTM disease from TB clinically and the fact that it is impossible to optimize specimen pre-treatment and incubation for more than 169 different NTM species makes it a reasonable routine that may – however - underestimate NTM [23,26,34,39-49].

Since molecular biological methods offer improved speed and accuracy in NTM identification, they have replaced conventional ones. Identification of NTM is mainly carried out by a commercial line assay supplemented with sequencing. The use of the same commercial method (Table 2, GenoType) in Greece may help the standardization of NTM identification and supports the comparison of results between the studied areas, but it also creates some vulnerability in case of quality problems of the commercial kit. Additionally, a significant percentage of all NTM isolates could not be recognized as valid species. In laboratories using commercial DNA probes this group consists of all strains that cannot be identified by commercial DNA probes. Consequently, new probes should be designed and added to the strip [5,26,63-67].

Different NTM species were detected most frequently in each of the NTM research studies in Greece (Table 1). M. gordonae, M. fortuitum, M. fortuitum – M. chelonae complex, M. avium and M. peregrinum were detected most frequently according to Crete study published at 2006. The order of the most frequently reported species changes when data from the same study area for the period 2000-2009 is taken into consideration. Now, M.
Conclusion

NTM diseases belong to the group of orphan diseases. Over the last few years, a growing number of NTM isolates, often of newly described species, are being submitted to laboratories for identification. The clinical relevance of these NTM is under constant evaluation. An early identification may lead to the institution of faster treatment and a better prognosis.

Still, the demographic changes might probably lead to a further increase in the incidence of NTM diseases in industrialized regions of Europe as well as worldwide. At present, clinical decisions mainly rely on expert opinion rather than on good clinical evidence. Care of affected patients can only be improved if a number of research priorities are recognized and addressed and furthermore, better evidence for the management of NTM diseases is generated by basic and clinical research. This mainly requires the collaboration of many colleagues to generate reliable data.

The prevention of NTM colonization and the elimination of these pathogens from infection sources are critical issues that must be solved rapidly. Investigations of the ecology of NTM species in environment and their sources and routes of infection, as well as the development of new and effective elimination methods, including new disinfectants and new medical treatment, are urgently needed.

Therefore, to prevent and treat NTM diseases, it is essential to identify the infection sources for these organisms, because patients with these diseases often suffer from reinfections and recurrent infections that co-exist with them.

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References


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