Nontuberculous Mycobacteria in a Tertiary Hospital in Portugal: A Clinical Review

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Abstract

Background: Nontuberculous mycobacteria (NTM) form a heterogeneous group regarding their ability to cause disease. To further understand their clinical relevance, the characteristics of patients who had positive cultures for NTM at a tertiary hospital in Portugal were reviewed.

Methods: Retrospective analysis of patients assessed at the Infectious Diseases (ID) Department of the São João Hospital Center, from January 2007 to December 2014, from whom at least one biological sample was tested culture positive for NTM.

Results: A total of 74 patients with at least one positive culture for NTM were identified. Forty-nine (66.2%) were infected by the human immunodeficiency virus, 4 (5.4%) had cancer, and 7 (9.5%) were under immunosuppressive medication. A total of 13 patients (17.6%) fulfilled the American Thoracic Society/ID Society of America criteria for pulmonary NTM disease and treatment was initiated in 12 other patients (16.2%), all of which were immunocompromised. Mycobacterium avium complex was more frequently associated with disease, responsible for 56% of the patients treated. Patients were treated with antituberculosis drugs adjusted for the species isolated, and cure was achieved in 13 patients (52%).

Conclusion: The present study highlights the importance of understanding the epidemiology of NTM to better comprehend their clinical impact.

Keywords: Colonization, epidemiology, immunosuppression

INTRODUCTION

Nontuberculous mycobacteria (NTM) are widely dispersed in our environment. Normal individuals are colonized by these organisms starting in childhood, and exposure increases with age.¹ Infection is normally acquired through contact with contaminated environment, with the possibility of human-to-human transmission virtually excluded.²⁻⁵ Over 170 species have been described to date, with the total number of NTM continuing to grow due to advances in microbiological and molecular techniques.⁶⁻⁷ At São João Hospital Center (SJHC), a tertiary hospital in Portugal, NTM have been reported to correspond to approximately 40% of the total mycobacteria strains identified.⁸ These species form a very heterogeneous group regarding their spectrum of virulence and their ability to cause disease, and their clinical relevance is not always completely understood. Most of them are not pathogenic for healthy individuals, but almost all can be responsible for opportunistic infections in susceptible individuals, making it difficult to distinguish colonization from true disease.⁹ Another obstacle in understanding NTM infection is its poorly defined epidemiology although evidence suggests an increase in prevalence in the past decades.¹⁰,¹¹ This is probably due to the microbiological advances leading to a more rapid and accurate identification of NTM but also due to a more susceptible population through aging and increases in immunosuppressive medication use and comorbidities such as diabetes mellitus and chronic obstructive pulmonary disease (COPD).¹² To further understand the epidemiology of NTM and their clinical relevance, the demographic and clinical characteristics of patients who had positive cultures for NTM at SJHC from 2007 to 2014 were reviewed.

METHODS

Study population

Data were retrospectively from patients assessed at the Infectious Diseases (ID) Department of SJHC, from January

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2007 to December 2014, from whom at least one biological sample was tested culture positive for NTM. SJHC is a tertiary care university hospital located in Porto, Portugal. Demographic, clinical, and radiographic data of the patients were collected. The study was approved by the ethics committee of SJHC.

**Specimen processing, acid-fast bacilli smear, and culture**

The clinical samples were decontaminated using the N-acetyl-L-cysteine-sodium hydroxide method and concentrated by centrifugation. The processed sediments were stained using the Auramine method and inoculated into MGIT™ 960 medium (Becton, Dickinson and Company (BD), New Jersey, USA), supplemented with BBL™ MGIT™ OADC (BD) enrichment and BBL™ MGIT™ PANTA™ antibiotic mixture (BD) for 42 days at 37°C, in accordance with the manufacturer’s instructions. Cultures were incubated in the automated culture system BACTEC™ MGIT™ 960 (BD) and monitored continuously for an advanced fluorometric technology which permits highly accurate detection of O₂ consumption. The cultures exhibiting growth in this automated system (manufacturer-set threshold: 75 Growth Units) were examined by smear microscopy by Kinyoun’s stain to examine acid-fast bacilli (AFB), before being considered positive.

**Mycobacterial molecular identification**

Positive cultures were then identified at the species level through GenoType Mycobacterium CM/AS (Hain Lifescience GmbH, Nehren, Germany), a molecular assay based on polymerase chain reaction (PCR) and the DNA-STRIP technology. In this method, a multiplex PCR with biotinylated primers is performed, followed by reverse hybridization and addition of a streptavidin/alkaline phosphatase conjugate. The staining reaction is mediated by an alkaline phosphatase. Three controls (conjugate, universal, and genus) are included in each strip.

**Statistical analysis**

SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) was used on data analysis.

### Results

A total of 74 patients with at least one positive culture for NTM were identified. Of the studied population, 81.1% (n = 60) were male with a median age of 46 years (range 26–84 years). The majority of the isolates (47%, n = 35) were recovered from respiratory samples, of which only 7 (20%) were AFB smear positive. In 3 of those patients, culture was positive only in bronchial or bronchoalveolar lavage, with negative sputum culture. The remaining isolates were obtained from gastric fluid (20.3%, n = 15), urine (10.8%, n = 8), fecal samples (8.1%, n = 6), tissue biopsy (4%, n = 3), blood samples (2.7%, n = 3), and cerebrospinal fluid (1.3%, n = 2). In four patients (5.4%), culture was positive for NTM in more than one site.

Out of the 74 patients, 49 (66.2%) were infected by the human immunodeficiency virus (HIV), 4 (5.4%) had cancer, and 7 (9.5%) were under immunosuppressive medication. Thirty-three (44.6%) were current or ex-smokers, 13 (17.6%) had a diagnosis of COPD, and 7 (9.5%) had bronchiectasis. Eleven (14.9%) of them had a previous tuberculosis (TB) infection.

The most common species isolated were *Mycobacterium avium* complex (MAC) (35.1%, n = 26), followed by *Mycobacterium gordonae* (28.4%, n = 21). In total, 9 different species were isolated. One isolate was identified as *Mycobacterium spp.*, but it was not possible to identify the species. In 10 patients (13.5%), there was a simultaneous diagnosis of TB with a positive culture for *Mycobacterium tuberculosis* in another sample and these patients were treated for TB and considered colonized by NTM. The isolates obtained are described in Table 1.

A total of 13 patients (17.6%) fulfilled the current American Thoracic Society/ID Society of America (ATS/IDSA) criteria for pulmonary NTM disease and 12 other patients (16.2%) were considered to have clinical evidence of NTM disease, all of which were immunocompromised (10 had HIV infection and 2 were under immunosuppressive medication). In total, 25 patients (33.8%) presented with NTM disease. The clinical characteristics of the patients are summarized in Table 2.

### Table 1: Nontuberculous mycobacteria isolated in infected and colonized patients

|                  | All (n=74), n (%) | Colonized (n=49), n (%) | Diseased (n=25), n (%) | P     |
|------------------|------------------|-------------------------|------------------------|-------|
| MAC              | 26 (35)          | 12 (24)                 | 14 (56)                | 0.007 |
| *Mycobacterium avium* | 19 (26)          | 7 (14)                  | 12 (48)                | 0.002 |
| *Mycobacterium intracellulare* | 7 (9)          | 5 (10)                  | 2 (8)                  | 1.000 |
| *Mycobacterium gordonae* | 21 (28)         | 19 (39)                 | 2 (8)                  | 0.005 |
| *Mycobacterium chelonae* | 6 (8)           | 3 (6)                   | 3 (12)                 | 0.400 |
| *Mycobacterium kansasii* | 6 (8)           | 4 (8)                   | 2 (8)                  | 1.000 |
| *Mycobacterium peregrinum* | 5 (7)           | 5 (10)                  | 0                      | 0.160 |
| *Mycobacterium xenopi* | 5 (7)           | 3 (6)                   | 2 (8)                  | 1.000 |
| *Mycobacterium fortuitum* | 3 (4)        | 2 (4)                   | 1 (4)                  | 1.000 |
| *Mycobacterium genavense/Mycobacterium triplex* | 1 (1)          | 0                       | 1 (4)                  | 0.338 |
| *Mycobacterium spp.* | 1 (1)           | 1 (2)                   | 0                      | 1.000 |

MAC: *Mycobacterium avium* complex
Table 2: Clinical and demographic characteristics of the studied population

| All (n=74), n (%) | Colonized (n=49), n (%) | Diseased (n=25), n (%) | P      |
|------------------|------------------------|------------------------|--------|
| Male             | 60 (81)                | 38 (76)                | 22 (88) | 0.358 |
| Age (median)     | 46 (26-84)             | 43 (27-84)             | 46 (26-66) | 0.567 |
| HIV positive     | 49 (66)                | 30 (61)                | 19 (76) | 0.299 |
| >200 CD4/mm²     | 9 (12)                 | 8 (16)                 | 1 (4)  | 0.258 |
| <200 CD4/mm²     | 40 (54)                | 22 (45)                | 18 (72) | 0.027 |
| <50 CD4/mm²      | 26 (35)                | 10 (20)                | 16 (64) | <0.001 |
| COPD             | 13 (18)                | 11 (22)                | 2 (8)  | 0.197 |
| Bronchiectasis   | 7 (9)                  | 3 (6)                  | 4 (16) | 0.217 |
| Current or ex-smoker | 33 (45)             | 23 (47)                | 10 (42) | 0.627 |
| Cancer           | 4 (5)                  | 4 (8)                  | 0      | 0.293 |
| Immunosuppressive medication | 7 (9)             | 4 (8)                  | 3 (13) | 0.682 |
| Previous tuberculosis | 11 (15)            | 10 (20)                | 1 (4)  | 0.083 |
| Over 65 years of age | 6 (8)                   | 4 (8)                  | 2 (8)  | 1.000 |

COPD: Chronic obstructive pulmonary disease, HIV: Human immunodeficiency virus

In HIV infected patients, there is a correlation between CD4 count and risk of infection (OR 0.985, P = 0.023). No statistically significant differences were identified between colonized and diseased patients in terms of demographic or clinical characteristics, except for HIV patient with CD4+ T lymphocyte bellow 50/mm², which were associated with NTM disease. In HIV-infected patients, there is a correlation between CD4 count and risk of infection (OR 0.985, P = 0.023).

Out of the 25 patients with NTM disease, 19 (76%) had HIV infection. Of these patients, only 6 were taking antiretroviral therapy, and almost all of them presented with severe immunodeficiency with a median CD4+ T lymphocyte count of 9 (range 1–265). Of the remaining 6 patients (24%), five were under immunosuppressive medication and the other one was splenectomized and had a diagnosis of COPD. In the patients considered to have NTM disease, MAC infection predominated with 14 of the 25 cases (56%), whereas in the patients considered to be colonized, M. gordonae was the most frequently isolated species (39%, n = 19).

Regarding clinical presentation, the most frequently reported symptoms were cough (68%, n = 17), weight loss and fatigue (64%, n = 16), anorexia (56%, n = 14), dyspnea (40%, n = 10), and fever (32%, n = 8). Twelve patients (48%) presented with pulmonary disease, and eleven patients (44%) were considered to have disseminated disease either by isolation of NTM in a blood culture, isolation of NTM in more than one site, or by clinical and/or radiological evidence of disseminated disease. One patient had cutaneous infection by Mycobacterium chelonae, and one patient had ganglionar infection by Mycobacterium intracellulare. Radiological features of the 23 patients with pulmonary or disseminated disease were as follows: 5 patients had unilateral consolidation, 7 had bilateral consolidation, 3 had cavitory disease, 3 had a nodular pattern, and 3 had ground-glass opacification. Five patients had a normal X-ray with radiological features only present in the computed tomography (CT) scan, and only 2 patients had a normal X-ray and CT scan.

Fifteen patients (60%) were initially treated with a quadruple anti-TB regimen (Isoniazid, Rifampin, Pyrazinamide, and Ethambutol [HRZE]). The treatment was adjusted once the diagnosis of NTM disease was made. MAC disease was treated with rifabutin, ethambutol, and macrolide (clarithromycin or azithromycin). The patients infected by M. chelonae were treated with clarithromycin. M. gordonae was treated with levofoxacin and azithromycin in one patient and levofoxacin, azithromycin, and ethambutol in the other patient. Mycobacterium kansasii infection was treated with HRZE, and Mycobacterium xenopi infection was treated with isoniazid, rifabutin, ethambutol, and clarithromycin. The patient with Mycobacterium fortuitum was treated with doxycycline and levofoxacin, and the patient with Mycobacterium genavense/triplex was treated with rifabutin, ethambutol, and clarithromycin. In 3 patients, the diagnosis was made postmortem and no treatment was initiated.

In 13 patients (52%), cure was achieved with no evidence of relapse. Seven patients (28%) died, all of them from causes related to severe immunosuppression. Five patients (20%) were lost to follow-up either by nonadherence or by transfer to another hospital.

**DISCUSSION**

The prevalence of NTM isolates seems to be rising in the past decades, while its clinical importance still remains controversial. Due to an aging population with growing comorbidities and news forms of immunosuppression, mycobacteriosis are becoming increasingly important. However, at ID departments, because the population is usually mostly HIV positive, the incidence of NTM infection was expected to decrease following the advent of highly active antiretroviral therapy (HAART), which seems to be the trend in our department [Table 3].

The isolation frequency of NTM species varies according to geographical region, clinical sample obtained, and medical history of the patient. A recent epidemiological
study performed in Portugal reported MAC, *M. kansasii*, *M. gordonae*, *M. xenopi*, and *M. fortuitum* as the most frequently isolated species responsible for NTM disease,\(^{20}\) data in line with our results. MAC has been consistently reported as the most frequently isolated NTM\(^{5,14,15,21}\) as was the case in this study. MAC was also the most clinically relevant isolate, as other epidemiological studies confirm.\(^{5,14,15}\) It was more frequently associated with disease as opposed to *M. gordonae* and *Mycobacterium peregrinum* which were more frequently associated with colonization.

Almost all of the patients presenting with NTM infection had some form of immunosuppression. Mycobacteriosis in HIV-infected patients was almost limited to those with a lymphocyte T CD4+ count below 200/mm\(^3\), with only one patient with a higher count of 265 lymphocyte T CD4+/mm\(^3\). This patient presented with ganglionar infection by *M. intracellulare*. With the introduction of HAART, severe immunosuppression has become increasingly rarer in the HIV-positive population, which indicates that other forms of immunosuppression (aging, diabetes, cancer, immunosuppressive medication, etc…) will probably gain more importance in the epidemiology of NTM infection.

The clinical presentation of patients with pulmonary NTM infection is very much similar to the clinical presentation of TB with nonspecific symptoms such as cough, weight loss, and fatigue, which were the most frequently reported symptoms in this study. Furthermore, X-ray changes may not always be present, and because of that, pulmonary CT scan becomes crucial in the diagnosis of patients with a high level of suspicion. Radiological presentation is inconsistent, with unilateral or bilateral consolidation being the most frequent finding. Cavitary disease, nodular pattern, or ground-glass opacification may also occur. Among the 12 patients with pulmonary disease, 3 of them had positive culture exclusively in bronchial or bronchoalveolar lavage with negative sputum culture, highlighting the importance of bronchofibroscopy to collect respiratory specimens for mycobacterial culture in some patients.

Out of the 25 patients with NTM disease, 23 (92%) had pulmonary disease (either localized or disseminated) but only 13 (56%) fulfilled the current ATS/IDSA criteria for pulmonary NTM disease,\(^{14}\) a lower number than expected. In a study done at the Pneumology Department at SJHC, 89% fulfilled the ATS/IDSA criteria, showing that these criteria are more adapted do Respiratory/Pneumology Department.\(^{8}\) These findings reinforce the need to adapt these criteria to severely immunosuppressed patients.

Although the incidence of TB in Portugal has been decreasing in the past few years, it is still considered a medium incidence country.\(^{22}\) As such, anti-TB drugs are usually initiated immediately after test results indicate an infection due to mycobacteria, with posterior adjustment after identification of the NTM species. Antibiotics used include aminoglycosides, fluoroquinolones, and macrolides. Patients treated for NTM should be closely monitored during treatment for side effects, namely hepatotoxicity. Combination therapy is usually required to prevent the development of resistance, with the exception of *M. chelonae* infection which has been shown to be susceptible to macrolide monotherapy. Both patients with pulmonary *M. chelonae* infection and the patient with cutaneous infection were treated successfully with clarithromycin. The declining incidence of TB in Portugal may as well play a part in the rising frequency of NTM infection, as some authors suggest a decline in cross-protective mycobacterial immunity associated with *M. tuberculosis* infection.\(^{23}\)

**Prognosis** is largely dependent on the degree of immunosuppression and comorbidities. In this study, mortality was high (28%) but limited to patients with severe immunosuppression. Almost all of them were HIV-positive, with only one HIV-negative patient who was under immunosuppressive therapy for rheumatoid arthritis and died of septic shock.

This study has the obvious limitations of being a retrospective study and analyzing data collected from patients assessed at an ID department, making it not representative of the general population. As such, HIV-infected patients are probably overrepresented as well as other forms of immunosuppression.

**Conclusion**

Mycobacteria form a very heterogeneous group, and some species are clearly more clinically relevant than others. In this study, MAC was more frequently associated with disease and *M. gordonae* with colonization. Through aging of the population, new forms of immunosuppression, and the advent of HAART for HIV patients, their distribution seems to be shifting. The present study highlights the importance of understanding the epidemiology of NTM to better comprehend their clinical impact. Surveillance and reporting systems of NTM isolates, similar to those in place for TB, are crucial to solve these questions and enlighten the medical community on the clinical importance of these microorganisms.

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**Table 3: Frequency of isolation of nontuberculous mycobacteria from 2007 to 2014**

| Year | Colonization | Infection |
|------|--------------|-----------|
| 2007 | 1            | 2         |
| 2008 | 3            | 4         |
| 2009 | 4            | 5         |
| 2010 | 5            | 6         |
| 2011 | 6            | 7         |
| 2012 | 7            | 8         |
| 2013 | 8            | 9         |
| 2014 | 9            | 10        |
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Conflicts of interest
There are no conflicts of interest.

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