Nontuberculosis Mycobacteria at a Multiresistant Tuberculosis Reference Center in Bahia: Clinical Epidemiological Aspects

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Objective: Examine the prevalence and clinical/epidemiological aspects of patients with nontuberculous mycobacteria (NTM) isolated from sputum provided by an outpatient clinic specializing in the treatment of multiresistant tuberculosis (MRTB) in Bahia, Brazil. Methods: All patients followed at the MRTB outpatient clinic of the Octávio Mangabeira Specialized Hospital (HEOM) were evaluated retrospectively from July 1998 to July 2003. All patients underwent direct examinations and cultures to identify the mycobacteria species found during initial and subsequent evaluations. The following variables were recorded: age, gender, clinical symptoms and signs, pre-existing lung disease, prior TB treatment, HIV serology, and NTM species. Categorical and quantitative variables were respectively characterized using proportions and measures ± SD. Results: NTM were isolated in 19 of 231 patients (8.2%; 95%CI: 5.2%-12.3%), with the following species distribution: 58% (11/19) *M. chelonae/abscessus;* 16% (3/19) *M. avium-intracellular* complex; 16% (3/19) *M. kansasii;* and 11% (2/19) *M. fortuitum.* HIV serology was positive for just one patient (5%), from whom *M. chelonae/abscessus* was isolated. Productive coughing was observed in all cases. American Thoracic Society (ATS) diagnostic criteria for NTM lung disease were observed in 14 patients (74%). Conclusions: The prevalence of NTM isolated from patients referred to the MRTB outpatient clinic in Bahia was 8.2% (CI 95%: 5.2%-12.3%); rapid-growth mycobacteria (*M. chelonae/M. fortuitum*) were the most frequently isolated (68%).

Key Words: Nontuberculous mycobacteria, lung disease, Brazil, Bahia.

Nontuberculous mycobacterial (NTM) diseases have become increasingly important in recent years, particularly due to their association with AIDS, cystic fibrosis and bronchiectasis, as well as improved clinical recognition and isolation of mycobacteria [1-15]. Although NTM can occur in several parts of the human organism, the most frequent localized clinical manifestation of NTM is lung disease [8,16-19]. The signs and symptoms of NTM lung disease are variable and non-specific, such as chronic productive coughing and fatigue. Other clinical manifestations, such as dyspnea (shortness of breath), hemoptysis (coughing up blood), fever and weight loss, can occur, particularly in cases of advanced lung disease [8]. The clinical presentation is frequently mistaken for coexisting lung diseases, which constitute conditions of risk for the development of NTM colonization and disease

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[8,17,19,20]. These conditions include complications of tuberculosis, bronchiectasis, cystic fibrosis, pneumoconiosis (especially silicosis), chronic obstructive pulmonary disease (COPD) and neoplasia [8,16,21,22]. Because NTM colonization of the respiratory tract can occur, particularly in patients with structural lung disease, it is necessary to determine whether the diagnostic criteria of the disease have been met, including clinical, radiological and bacteriological findings [8].

Although there has been a recent increase in the number of publications on NTM, few studies focus on HIV-seronegative target populations [16, 23, 24]. We examined the prevalence of NTM isolated from sputum and clinical/epidemiological aspects of patients from the multiresistant tuberculosis (MRTB) outpatient clinic in Bahia (Northeastern Brazil).

Material and Methods

Study location

Octávio Mangabeira Specialized Hospital (HEOM), a tertiary unit dedicated to the treatment of TB and other lung diseases, equipped with 180 beds. The HEOM outpatient clinic is the only reference center for MRTB in Bahia accredited by the Ministry of Health, and it treats patients originating from the entire health system who have been referred due to a suspected or confirmed diagnosis of MRTB.

Population studied

236 patients referred to the HEOM clinic with suspected MRTB between July 1998 and July 2003.

Routine procedures for patients with suspected MRTB

All patients were evaluated by the same medical team, who carried out the following tests: 1) direct testing for acid-alcohol resistant bacilli in sputum using the Ziehl-Neelsen method; 2) Lövenstein-Jensen culture of mycobacteria from sputum; 3) biochemical typing of mycobacteria isolated from culture; 4) testing sensitivity to anti-TB drugs using the proportion method; 5) HIV serology; 6) chest X-rays (posterior-anterior and lateral). All cultures and acid fast bacilli (AFB) smears were performed at the HEOM mycobacteriology lab. HIV serology, mycobacteria typing and sensitivity testing were carried out at the Gonçalo Muniz Central Laboratory (LACEN-Bahia). All patients were evaluated on a monthly basis.

Research variables of interest

The medical records of patients with NTM isolated from sputum were reviewed retrospectively. The variables of greatest interest were 1) demographic: age, gender and skin color; 2) clinical: symptoms, such as coughing, expectoration, fatigue, fever, weight loss, hemoptysis, enlarged lymph nodes; 3) epidemiological: history of TB treatment, pre-existing lung disease, HIV/NTM co-infection, place of origin (rural or urban); bacteriological: mycobacteria species isolated, number of direct tests and positive cultures, quantification of mycobacteria colonies cultured (colony count, from 1 to 3), 5) radiographic: determining changes in chest X-rays compatible with NTM disease or structural lung disease – complications of pulmonary tuberculosis (PTB), bronchiectasis, silicosis, DPOC, etc.). American Thoracic Society (ATS) criteria were used to evaluate the clinical significance of isolated NTM to determine the presence of NTM lung disease, based on clinical, bacteriological and radiological findings [8] (Table 1).

Statistical analysis

The statistical analysis was basically descriptive, using SPSS 9.0 software. Categorical and quantitative variables were respectively characterized using proportions and averages ± standard deviations. The accuracy of random estimates was described through a confidence interval of 95% (95%CI), utilizing PEPI statistical software.

Results

Five of the total of 236 patients referred to the HEOM reference outpatient clinic with a suspected diagnosis of MRTB had a contaminated initial culture that prevented the identification of mycobacteria. Of the 231 patients with available culture results, *Mycobacterium tuberculosis* was isolated from 212 (91.8%) and NTM from 19 (8.2%; 95%CI: 5.2%-12.3%).

The characteristics of NTM patients are shown in Table 2. The average age was 48.8 ± 13.8 years, and patients were predominantly male (68.4%). Regarding skin-color, 42% (8/19) were white and 58% (11/19) were brown. Seventeen of the 19 patients (90%) were of urban origin. Only one NTM patient was HIV seropositive (5%). Tobacco and alcohol use were observed in 32% and 26% of the patients, respectively. All of the 19 patients had a history of prior TB treatment, with a diagnosis based solely on an AFB smear. Pre-existing lung disease was found in 79% (15/19) of the patients in the sample (Table 2).

The NTM species most frequently isolated in this series was *M. clelonae/abscessus*, which was found in 11 of the 19 patients (58%). The other three NTM species identified were *M. avium-intracellular* in 16% (3/19), *M. kansasii* in 16% (3/19) and *M. fortuitum* in 11% (3/19). Rapid-growth NTM (*M. clelonae/abscessus* and *M. fortuitum*) represented 68% (13/19) of all species isolated (Table 3). Five of the 19 patients had just one NTM-positive culture, and this finding was interpreted as colonization. The remaining 14 patients had two or more positive cultures for the same NTM species (Table 4).

The signs and symptoms presented by these patients are summarized in Table 5. The most frequent symptom was productive coughing, which was present in all patients. Eleven of the 19 patients (58%) reported shortness of breath in the initial evaluation. Fatigue was observed in 26%, hemoptysis in 21%, chest pain in 21% and fever in 16%. Extra-pulmonary clinical manifestations, such as enlarged lymph nodes and skin lesions, were not observed in this series.

The results of chest X-rays are shown in Table 6. The most frequent radiological findings were: atelectasis with cavitation (38.9%); infiltrates with or without nodules (27.7%); and small multiple nodules (22.2%). The basic disease in three of the four patients with small multiple nodules was silicosis. High-resolution chest CAT scans were performed on two patients, showing the presence of bronchiectasis. One of these female patients with diffuse bronchiectasis had cystic fibrosis and the other not only had localized bronchiectasis in the lower lingular lobe, but she also had nodules and a bronchial tree pattern compatible with *M. avium-intracellular* infection, confirmed by the repeated isolation of this species from sputum.

Fourteen of the 19 patients (74%) met ATS diagnostic criteria for NTM disease [8]. The 14 patients with NTM disease received appropriate treatment for the species identified. The two patients infected with *M. kansasii* received a four-drug regimen (RISE – rifampin, isoniazida, ethambutol and streptomycin) for a period raging from 9 to 12 months. One was cured and another developed extensive destruction of pulmonary parenchyma, with persistence of a positive sputum, leading to death. All three patients from whom *M. avium-intracellular* was isolated met the criteria for diagnosing the disease. Two received a regimen of macrolide antibiotics (clarithromycin in one patient and azithromycin in another) associated with ethambutol and amikacin, and cure was obtained in both cases. One patient with *M. avium* infection was unable to maintain the treatment with a macrolide regimen. Therefore, ofloxacin, amikacin and ethambutol were used, with an unfavorable response, leading to death due to respiratory insufficiency. Nine of the 13 patients with rapid-growth NTM (*M. chelonae/abscessus* and *M. fortuitum*) were considered to be carriers of the disease. Five received a mixed regimen of macrolide antibiotics (clarithromycin or azithromycin), ofloxacin and amikacin. The other four were unable to maintain the macrolide regimen (due to an irregular supply of the drug in the public health system), which was replaced with clofazimine. Three of the 9 rapid-growth NTM patients were cured, three showed treatment failure with persistent positive cultures, two cases evolved to death (not related to NTM disease, but due to

Table 1. Diagnostic criteria for nontuberculous mycobacterial (NTM) lung disease*

Criteria	
1. Clinical	a. Compatible signs and symptoms (coughing, fatigue more common; weight loss, hemoptysis and shortness of breath may be present, particularly in advanced disease) with documented deterioration of the patient's clinical state if a base condition is present and b. Reasonable exclusion of other diseases (e.g., tuberculosis, cancer, histoplasmosis) that could explain the condition, or adequate treatment of the other condition when signs/symptoms increase
2. Radiological	a. Any of the following changes in the chest X-ray; if a previous X-ray was taken over a year before, evidence of progression must be found · Infiltrates with or without nodules (persistent ≥ 2 months or progressive) · Cavitation · Only nodules (multiple) b. Any of the following changes in chest CAT scan · Multiple small nodules · Multifocal bronchiectasis with or without small nodules
3. Bacteriological	a. In at least 3 sputum/bronchial wash samples available within 1 year · Three positive cultures with negative AFB smear results or · Two positive cultures and one positive AFB smear result or b. One bronchial wash and unavailability of sputum samples · Positive culture with 2+, 3+, or 4+ growth on solid media C or · Positive culture with 2+, 3+, or 4+ AFB smear or c. Tissue biopsy · Any growth in transbronchial biopsy · Granulomatous inflammation and/or positive AFB smear in lung biopsy with one or more positive cultures of sputum/bronchial wash · Any growth in a sterile extrapulmonary site

* Table adapted from American Thoracic Society – Diagnosis and treatment of disease caused by nontuberculous mycobacteria, 1997.

Table 2. Demographic and epidemiological characteristics of patients with NTM isolates in respiratory secretions

Characteristics	N=19
Age in years (average ± SD)	48.8 (13.8)
Male, N(%)	13 (68)
Skin-color, N (%)	
White	8 (42)
Brown	11 (58)
Origin, N(%)	
Urban	17 (90)
Rural	2 (11)
HIV serology, N(%)	
Positive	1 (5)
Negative	18 (95)
Tobacco use, N (%)	6 (32)
Alcoholism, N(%)	5 (26)
Prior treatment for TB, N (%)	19 (100)
Pre-existing lung disease, N (%)*	18 (95)
TB complications	11 (58)
Bronchiectasis	1 (5)
Silicosis	3 (16)

* Some patients had more than one pre-existing disease.

Table 3. Percentage distribution of NTM species isolated from sputum (N=19)

NTM species	N (%)
Mycobacterium chelonae / abscessus	11 (58)
M. avium-intracelullar	3 (16)
M. kansasii	3 (16)
M. fortuitum	2 (11)

Table 4. Number of NTM-positive cultures isolated from sputum (N=19)

Number of positive cultures	N (%)
1 culture	5 (26)
2 cultures	6 (32)
3 cultures	6 (32)
4 cultures	1 (5)
5 cultures	1 (5)

Table 5. Frequency of signs and symptoms of patients with NTM isolated from sputum (N=19)

Signs/symptoms	N (%)
Productive coughing	19 (100)
Shortness of breath	11 (58)
Fatigue	5 (26)
Hemoptysis	4 (21)
Chest pain	4 (21)
Fever	3 (16)

Table 6. Frequency of changes in NTM patients' chest X-rays (N=18)ψ

Radiological findingsφ	N (%)
Infiltrate with cavitation**	2 (11)
Small nodules (multiple)*	4 (22)
Infiltrate with or without nodules·	5 (28)
Atelectasis with cavitation**	7 (39)
Bronchiectasis***	2 (11)

ψ Chest X-rays for one patient could not be located. φ Some patients presented more than one of the radiological findings described.
* Three cases with associated silicosis. ** All cases compatible with TB complications. *** One case of a cystic fibrosis patient and one case of lingular bronchiectasis. secondary to *M. avium-intracellular.*
· Two cases compatible with TB complications.

complications of the basic disease – AIDS in one case and cystic fibrosis in the other) and one patient abandoned treatment, so follow-up was lost.

Discussion

We found that the prevalence of NTM isolated from the sputum of patients from the specialized MRTB clinic in Bahia was significant (8.2%), taking into account the high prevalence of TB in that region. There are three plausible explanations for this finding: (1) the MRTB reference center receives patients who do not respond to conventional TB treatment, and have persistently positive AFB smears and/or cultures; (2) there is a dearth of systematic investigation of mycobacteria species in our state's laboratories; and (3) professionals at basic units that treat TB patients are unfamiliar with the diagnosis and therapeutic management of NTM lung disease.

In agreement with our findings, Shanker et. al. found a 7.9% prevalence of NTM in 604 mycobacteria-positive cultures from patients at a TB clinic in India [25]. In a study of patients admitted to the TB ward of a Canadian hospital, Goldstein et al. reported that the prevalence of NTM was 10% [26]. Other authors have observed a higher prevalence in samples of respiratory specimens from patients at hospitals and respiratory clinics [18,19,27,28]. Other studies conducted in Brazil have shown an even higher prevalence than that found in our study. In Araraquara, São Paulo (southeastern Brazil), Leite et. al. found a prevalence of 11.5% [29] and in Manaus (northern Brazil), Salem et al., found it to be 25.4% [30]. However, these differences may be attributed to the geographic distribution of NTM throughout Brazil, which is greater in the northern part of the country. Two other Brazilian studies carried out in a tertiary reference hospital for AIDS in Rio de Janeiro (southeastern Brazil) showed NTM prevalences of 5.8% (18/313) [31] and 15% (35/233) [5]. The high

prevalence observed in this latter study was due to the fact that mycobacterial cultures were performed for respiratory specimens and specimens from other sites. Furthermore, the NTM isolated from non-respiratory sites were from HIV-positive patients. There are several possible explanations for the varying estimates of the prevalence of NTM in patients treated at TB units, including the influence of the geographic and temporal distribution of NTM and the prevalence of TB and HIV infection in the population. Another possibility that could justify these findings is differences in the ages of the populations studied, as older people tend to have more pre-existing lung disease, and this is an important risk factor for NTM colonization and/or infection.

In this series, rapid-growth NTM (*M. chelonae/abscessus* and *M. fortuitum*) were observed in 68% (13/19) of the patients. This finding differs from those of many published studies, where *M. avium-intracellular* was the most frequently found [2,5,16,17,19,21,32-36]. However, some studies have found a higher frequency of other NTM species, such as *M. gordonae* [18], *M. xenopi* [27], *M. fortuitum*, *M. kansasii* [23] and *M. chelonae* [22]. In a study made of cultures sent to a Brazilian reference lab for mycobacteria, Barreto et al. reported a prevalence of approximately 44% *M. avium* (n=590) [33]. Nevertheless, when the regional distribution of isolates was examined, rapid-growth NTM (*M. chelonae* (13/70; 19%) and *M. fortuitum* (13/70; 19%) were isolated from 37% (26/70) of the cultures in the Brazilian northeast (including Bahia). The surprising predominance of rapid-growth NTM isolated from our series, particularly *M. chelonae/abscesssus*, therefore concurs with the findings of Barreto et al. in cultures from the northeastern part of Brazil [33]. *Mycobacterium avium* is known to be more prevalent in cases of HIV infection, and this could explain the results of our study, because there was only one HIV-positive patient in our series of cases. It is known that northeastern Brazil has a lower prevalence of HIV infection than in the south and southeast regions of the country.

Another aspect that should be taken into consideration is the presence of pre-existing chronic lung disease in approximately 80% of the patients in this series, which probably facilitated colonization and/or infection with less pathogenic strains of NTM, such as rapid-growth species (*M. chelonae* and *M. fortuitum*). Another plausible explanation could have to do with the geographic distribution of NTM species (which is plausible in Brazil, due to the country's vast size) and environmental factors [32, 33]. Rapid-growth NTM have been increasingly studied in recent years, due to their growing importance as a possible cause of lung disease [22,38-40].

This series was made up of patients with pre-existing lung disease, with TB complications being the most frequent (58%). Other co-morbidities presented by patients in this study, such as bronchiectasis and silicosis, are classically described as NTM risk factors [8,23,41]. *Mycobacterium chelonae* was repeatedly isolated from six cultures from a female patient in this series (5%) who had cystic fibrosis. Several recent studies have focused on cystic fibrosis as an important condition associated with a high frequency of NTM isolation [9-15, 35, 36, 42-47]. In a multicentric study conducted in the USA (n= 986), Olivier et al. found a 13% prevalence of NTM in cystic fibrosis patients, *M. chelonae* being the second-most frequently found species [35]. However, the applicability of ATS criteria to distinguish the colonization of the disease in these patients is debatable, and the NTM that were isolated have a questionable impact on the prognosis of cystic fibrosis. [15, 35, 44, 46].

In our study the NTM that was isolated was clinically significant in 74% of the series, on the basis of ATS criteria [8]. Other studies have shown smaller frequencies of clinically significant isolates [1,16,19,22]. However, two studies conducted in Denmark (about 50%) and Singapore (approximately 63%) have reported data that are more in agreement with those of our series [21,34]. Among other explanations, this finding may be due to a selection bias, because all patients came from the MRTB reference clinic and had had several positive cultures for mycobacteria, but in most cases, the species had not been identified, and therefore they were considered to be infected with MRTB. In the studies that reported a low percentage of clinically significant isolates, participants were

selected via laboratory reports, and they formed populations with clinical characteristics that differed from those of this study.

In closing, we call attention to the fact that a significant percentage of clinically significant NTM isolated from sputum cultures was found in patients referred to the MRTB outpatient clinic. There was a predominance of rapid-growth mycobacteria, particularly *M. chelonae/abscessus.*

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