Original Article

Descriptive study of the frequency of nontuberculous mycobacteria in the Baixada Santista region of the state of São Paulo, Brazil*

Estudo descritivo da freqüência de micobactérias não tuberculosas na Baixada Santista (SP)

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Abstract

Objective: The present study aims at describing the frequency of nontuberculous mycobacteria (NTM) species identified through laboratory testing of samples collected from nonsterile sites (sputum), as well as its frequency in HIV-infected and non-HIV-infected individuals in the Baixada Santista region of the state of São Paulo, Brazil, in the period from 2000 to 2005. **Methods:** Retrospective analysis of sputum smear microscopy results and culture was conducted based on the records on file at the Instituto Adolfo Lutz-Santos, the regional tuberculosis laboratory. **Results:** We analyzed 194 NTM strains isolated from 125 individuals, of whom 73 (58.4%) were HIV-negative and 52 (41.6%) were HIV-positive. Thirteen different species were identified: *Mycobacterium kansasii*; *M. avium* complex; *M. fortuitum*; *M. peregrinum*; *M. gordonae*; *M. terrae*; *M. nonchromogenicum*; *M. intracellulare*; *M. flavescens*; *M. bohemicum*; *M. chelonae*; *M. shimoidei*; and *M. lentiflavum*. In 19.2% of the cases, the bacteriological diagnosis was confirmed by isolation of the same species in at least two consecutive samples. **Conclusions:** Our results show the importance of including systematic identification of NTM in the laboratory routine, and that its integration into the clinical routine could improve the characterization of the disease, thereby informing the planning of effective control measures in specific populations, such as individuals presenting tuberculosis/HIV co-infection.

Keywords: Mycobacteria, atypical; Laboratory techniques and procedures; HIV; Tuberculosis.

Resumo

Objetivo: Este estudo teve por objetivo descrever a freqüência das espécies de micobactérias não tuberculosas (MNT) identificadas laboratorialmente a partir do isolamento de sítios não estéreis (escarro) de indivíduos infectados ou não pelo vírus HIV na Baixada Santista (SP), período de 2000 a 2005. **Métodos:** Foi realizada análise retrospectiva dos resultados de baciloscopia e cultura, disponíveis nos registros do laboratório regional de tuberculose, Instituto Adolfo Lutz-Santos. **Resultados:** Analisou-se 194 cepas de MNT correspondentes a 125 indivíduos, sendo 73 (58,4%) HIV negativos e 52 (41,6%) HIV positivos. Foram identificadas 13 diferentes espécies: *Mycobacterium kansasii*; complexo *M. avium*; *M. fortuitum*; *M. peregrinum*; *M. gordonae*; *M. terrae*; *M. nonchromogenicum*; *M. intracellulare*; *M. flavescens*; *M. bohemicum*; *M. chelonae*; *M. shimoidei;* e *M. lentiflavum*. Em 19,2% dos casos obteve-se diagnóstico bacteriológico confirmado pelo isolamento da mesma espécie em no mínimo duas amostras consecutivas. **Conclusões:** Os resultados mostram a importância da realização sistemática da identificação de MNT na rotina laboratorial e sua integração com a clínica, podendo contribuir na caracterização da doença e ações de efetivo controle, como nas populações co-infectadas tuberculose e HIV.

Descritores: Micobactérias atípicas; Técnicas e procedimentos de laboratório; HIV; Tuberculose.

Introduction

The *Mycobacterium* genus is represented by species belonging to the *M. tuberculosis* complex, as well as *M. leprae* and others, that, despite normally being saprophytic, can become opportunistic pathogens and cause serious injuries. In the latter case, the microorganisms are currently known as nontuberculous mycobacteria (NTM).[1-3]

Over 120 NTM species have been identified, and approximately one third have been associated with diseases

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in humans.[4] The pathogenic potential of NTM species varies, and they can cause pulmonary or extrapulmonary infections, principally in susceptible or immunocompromised individuals, such as those co-infected with HIV.[1,3,5-7]

Studies have shown there is marked geographic variability in the prevalence and distribution of species responsible for NTM-related diseases.[3] In developed countries, the incidence of tuberculosis (TB) has decreased, whereas that of NTM-related pulmonary diseases has increased. The AIDS pandemic was the most relevant factor contributing to this increase.[8-11] Very little is known about the incidence of NTM among HIV-positive and HIV-negative individuals in developing countries. In Brazil, an increase in the incidence of NTM-related diseases has been found after the AIDS epidemic; however, the frequency of the species and the prevalence of the disease remain unknown.[12,13]

Due to the high prevalence of TB and TB/HIV co-infection in the *Baixada Santista* region of the state of São Paulo, Brazil, the objective of this study was to describe the frequency of NTM species, identified in clinical samples collected from HIV-negative and HIV-positive individuals treated at health care facilities in the region.

Adolfo Lutz Institute-Santos is the regional reference laboratory for the bacteriological identification of TB in the *Baixada Santista* region, receiving all of the samples for mycobacterium culture from the public health care system.

Methods

This was a retrospective study of the records regarding the routine laboratory testing of sputum samples collected from individuals suspected of having TB and sent to Adolfo Lutz Institute-Santos between January of 2000 and December of 2005.

Table 1 - Distribution of nontuberculous mycobacteria, according to HIV serology results, identified in the Baixada Santista region between 2000 and 2005.[a]

Identification	Strains	Individuals		
	n (%)	HIV-negative n (%)	HIV-positive n (%)	Total (%)
Mycobacterium kansasii	65 (33.6)	20 (16.0)	5 (4.0)	25 (20.0)
Complexo *M. avium*	33 (17.0)	10 (8.0)	13 (10.4)	23 (18.4)
M. fortuitum	26 (13.4)	14 (11.2)	6 (4.8)	20 (16.0)
SGNM	9 (4.6)	1 (0.8)	5 (4.0)	6 (4.8)
M. peregrinum	4 (2.1)	2 (1.6)	0 (0.0)	2 (1.6)
SGSM	4 (2.1)	0 (0.0)	3 (2.4)	3 (2.4)
M. gordonae	3 (1.6)	3 (2.4)	0 (0.0)	3 (2.4)
M. terrae	2 (1.0)	2 (1.6)	0 (0.0)	2 (1.6)
M. nonchromogenicum	2 (1.0)	1 (0.8)	1 (0.8)	2 (1.6)
FGNM	2 (1.0)	1 (0.8)	1 (0.8)	2 (1.6)
M. intracellulare	2 (1.0)	2 (1.6)	0 (0.0)	2 (1.6)
M. fortuitum e *M. chelonae*[b]	2 (1.0)	0 (0.0)	2 (1.6)	2 (1.6)
M. triviale e *M. terrae*[b]	2 (1.0)	0 (0.0)	2 (1.6)	2 (1.6)
M. tuberculosis e *M. kansasii*[b]	2 (1.0)	1 (0.8)	0 (0.0)	1 (0.8)
M. flavescens	1 (0.5)	1 (0.8)	0 (0.0)	1 (0.8)
M. bohemicum	1 (0.5)	0 (0.0)	1 (0.8)	1 (0.8)
M. chelonae	1 (0.5)	0 (0.0)	1 (0.8)	1 (0.8)
FGSM	1 (0.5)	0 (0.0)	1 (0.8)	1 (0.8)
M. shimoidei	1 (0.5)	0 (0.0)	1 (0.8)	1 (0.8)
M. lentiflavum	1 (0.5)	0 (0.0)	1 (0.8)	1 (0.8)
NTM	30 (15.6)	15 (12.0)	9 (7.2)	24 (19.2)
Total	194 (100)	73 (58.4)	52 (41.6)	125 (100)

SGNM: slow-growing nonchromogenic mycobacteria; FGNM: fast-growing nonchromogenic mycobacteria; SGSM: slow-growing scotochromogenic mycobacteria; FGSM: fast-growing scotochromogenic mycobacteria; and NTM: nontuberculous mycobacteria. [a]Source: laboratory registry of Adolfo Lutz Institute-Santos. [b]Mixed culture.

The following data, obtained from the laboratory registry of sputum smear microscopy and culture, were included in the study: name, gender, age, serologic testing for HIV, sputum smear microscopy result, culture result and NTM species identification.

Laboratory techniques (sputum smear microscopy and culture), standardized in accordance with norms and guidelines described in the Guidebook for Tuberculosis Bacteriology published by the Brazilian National Ministry of Health, were routinely used at the Adolfo Lutz Institute-Santos.[14-17] In this study, a strain was defined as a single species isolated in the culture of a sputum sample.

Confirmation of the laboratory testing diagnosis of NTM was based on the criterion established by the American Thoracic Society,[1] which recommends the isolation of the same species in three positive culture samples if sputum smear microscopy is negative, or in two positive culture samples if one sample tested positive in sputum smear microscopy, within a year. Therefore, two different techniques were employed.

Ziehl-Nielsen-stained smears were examined under microscopy for the semi-quantitative count of acid-fast bacilli and cord factor detection.[14]

For the isolation of mycobacteria, we used Lowenstein-Jensen agar culture medium and the automated MB/Bact® system (bioMérieux, Marcy l'Étoile, France).[14]

Phenotyping of strains, for presumptive identification, included macroscopic screening (colony morphology and pigmentation) and microscopic analysis (acid-fast bacilli morphology, cord factor detection and contaminating microorganisms). Strains with pigmented or smooth nonphotochromogenic colonies that showed no cord factor under microscopy were presumptively classified as NTM. These strains were then submitted to phenotyping (growth temperature, growth period, enzymatic tests and antibiogram). Strains with inconclusive results were classified according to their growth period and pigmentation, such as slow-growing nonphotochromogenic mycobacterium, slow-growing scotochromogenic mycobacterium, fast-growing nonphotochromogenicmycobacterium, fast-growing scotochromogenic mycobacterium and fast-growing photochromogenic mycobacterium.[15,18,19]

Molecular identification of species was carried out using C-reactive protein method and PCR-restriction enzyme analysis of a 441-pb fragment of the *hsp*65 gene (PRA-*hsp*65). The bacterial mass was boiled in ultrapure water for 10 min, after which the DNA was extracted. Subsequently, the DNA was amplified using Tb11 and Tb12 primers as described in a study published in 1993.[20] Amplified fragments were digested separately using *Bst*EII and *Hae*III enzymes, and the products were separated using electrophoresis on a 3% agarose gel. The restriction pattern was visually analyzed and compared to PRASITE for the determination of the species.[21]

Results

During the study period, 194 NTM strains were isolated from 125 individuals. Of the 194 strains isolated, 142 (76.2%) presented conclusive results, and 52 (26.8%) presented inconclusive results (Table 1).

Of the strains presenting inconclusive identification, 30 (15.5% of the 194 strains) presented negative results for *M. tuberculosis* complex, classified in this study as NTM, and 16 (8.2% of the 194 strains) were classified according to their growth period and pigmentation. Of the 194 strains, 6 (3%) were mixed cultures that, after isolation and separation of colonies with distinct morphologies, were identified as two different mycobacterium species. The most frequent NTM species were *M. kansasii* (33.6%), *M. avium* complex (17%) and *M. fortuitum* (13.4%), accounting for 64% of the total number of strains (Table 1).

Of the 125 individuals suspected of having mycobacteriosis, with ages ranging from 18 to 75 years, 94 (75.2%) were male. The 40-49 age bracket was the most prevalent (36.8%). Of the total number of individuals, 58.3% were HIV-negative, and 41.6% were HIV-positive.

Using the criteria established by the American Thoracic Society for the confirmation of NTM laboratory diagnosis,[1] those of 101 individuals (80.8%) were unconfirmed. Of the 24 individuals (19.2%) whose diagnosis was confirmed, 12 (50%) presented three or more samples with positive results for the same species, and 12 (50%) presented two samples with positive results for the same species and one positive sputum smear microscopy result within a year.

Discussion

As a rule, NTM are opportunistic agents for most individuals, being able to simply colonize immunocompetent individuals and to cause severe diseases in those who are immunocompromised. Therefore, it is necessary to establish safe criteria in order to differentiate colonization from infection. In addition to the establishment of clinical, epidemiological and radiological criteria for the definitive diagnosis of NTM, laboratory methods that provide the correct identification of species are necessary for patient treatment and follow-up.[7]

The first question for laboratory diagnosis is to determine whether the presence of these agents means mere colonization or disease. In this study, using the guidelines established by the American Thoracic Society for the laboratory diagnosis of NTM in nonsterile material (sputum),[1] we found that 80.8% of the individuals in our sample had only one positive culture result, and the diagnosis was therefore unconfirmed. This number is very similar to the 79.8% found in another study,[22] in which 1892 strains of pulmonary origin obtained from nonsterile sites between 1991 and 1997 were evaluated. This shows that there is still a lack of integration between clinical practice and laboratory testing, since there is no guarantee that there will be multiple isolations from which to determine the bacteriological diagnosis.

Although only 19.2% of the cases were considered conclusive for the laboratory diagnosis of NTM in the present study, this result can be considered favorable when compared to the abovementioned study,[22] in which only 7.8% of the cases were confirmed through isolation of the same species in three or more samples tested in the laboratory.

Of the 13 NTM species identified in the present study, we found a wide variety of mycobacteria: *M. kansasii*, *M. avium* complex, *M. fortuitum*, *M. peregrinum*, *M. gordonae*, *M. terrae*, *M. nonchromogenicum*, *M. intracellulare*, *M. flavescens*, *M. bohemicum*, *M. chelonae*, *M. shimoidei* and *M. lentiflavum*. We have identified this diversity of species due to the implementation of the PRA-*hsp*65 technique, a relevant tool for the identification of the recently described species that have clinical significance.

The most common species in HIV-negative patients were *M. kansasii* (16%) and *M. fortuitum* (11.2%), whereas the most common species in HIV-positive patients was *M. avium* complex (10.4%), which reinforces the data found by other authors regarding the frequency of these species in various regions.[3,8,13,18,22] Unlike the results of a study carried out in 2003,[23] comprising 72 cultures from HIV-positive and HIV-negative individuals suspected of having mycobacteriosis (*M. avium*, 5.6%; *M. kansasii*, 2.7%), the data regarding the frequency of NTM in the Baixada Santista region differ from those reported for other regions, in which *M. avium* was the most frequently isolated species.[13,22] In the present study, *M. kansasii* was the most common species, highlighting a problem that had not yet been detected in the region.

The diversity of NTM species found in the Baixada Santista region underscores the importance of including systematic identification of NTM in the laboratory routine, by means of standardized techniques. The identification of mixed cultures is equally important, since *M. tuberculosis* can be masked by other mycobacteria with similar morphology, jeopardizing the diagnosis and delaying treatment. Therefore, we find the interaction between laboratory testing and clinical practice opportune, in order to guarantee multiple isolations from the same site, contributing to the characterization of the disease and the planning of effective control measures, especially in populations with high prevalences of TB and TB/HIV co-infection.

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