Original Article

Nontuberculous mycobacteria isolated in São José do Rio Preto, Brazil between 1996 and 2005*

Isolamento de micobactérias não-tuberculosas em São José do Rio Preto entre 1996 e 2005

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Abstract

Objective: To study the incidence of nontuberculous mycobacteria and the range of species isolated between 1996 and 2005 at a regional branch of the Adolfo Lutz Institute—located in the city of São José do Rio Preto, Brazil—and to show the importance of laboratory testing. **Methods:** Mycobacteria were isolated from pulmonary and extrapulmonary specimens and identified through phenotyping and molecular methods (polymerase chain reaction-restriction enzyme analysis). **Results:** We isolated 317 nontuberculous mycobacterium strains: *Mycobacterium avium* complex, 182 (57.4%); *M. gordonae*, 33 (10.4%); *M. fortuitum*, 25 (7.9%); *M. chelonae*, 8 (2.5%); *M. terrae* complex, 8 (2.5%); *M. kansasii*, 7 (2.2%); and less frequent species, 54 (17%). During this period, 72 cases (33.3%) were characterized as mycobacteriosis, according to bacteriological criteria established by the American Thoracic Society in 2007. Of those 72 cases, 56 were attributed to *M. avium* complex. Of those 56, 29 (51.8%) were characterized as disseminated disease. Six cases were attributed to *M. fortuitum*, 3 to *M. gordonae*, 2 to *M. chelonae*, 1 to *M. abscessus*, 1 to *M. kansasii*, 1 to *M. intracellulare*, 1 to *M. malmoense* and 1 to *Mycobacterium* ssp. **Conclusions:** These results show the importance of the bacteriological diagnosis, since identification of the species enables early and appropriate treatment.

Keywords: Mycobacteria, atypical/isolation & purification; Mycobacteria, atypical/classification; Diagnostic techniques and procedures.

Resumo

Objetivo: Estudar a ocorrência de micobactérias não-tuberculosas e a variabilidade das espécies isoladas na região atendida pelo Instituto Adolfo Lutz—Regional de São José do Rio Preto—no período entre 1996 e 2005, assim como mostrar a importância do diagnóstico laboratorial. **Métodos:** A partir de amostras pulmonares e extrapulmonares, foi realizado o isolamento de micobactérias, e estas foram identificadas por métodos fenotípicos e pelo método molecular *polymerase chain reaction-restriction enzyme analysis*. **Resultados:** Foram isoladas 317 cepas de micobactérias não-tuberculosas: complexo *Mycobacterium avium*, 182 (57,4%); *M. gordonae*, 33 (10,4%); *M. fortuitum*, 25 (7,9%); *M. chelonae*, 8 (2,5%); complexo *M. terrae*, 8 (2,5%); *M. kansasii*, 7 (2,2%); e espécies menos freqüentes, 54 (17%). No período, foram caracterizados 72 casos (33,3%) de micobacterioses, de acordo com os critérios bacteriológicos estabelecidos pela *American Thoracic Society* (2007).Desses, complexo *M. avium* foi responsável por 56 casos, sendo que 29 (51,8%) foram caracterizados como doença disseminada. *M. fortuitum* foi responsável por 6 casos; *M. gordonae*, 3; *M. chelonae*, 2; *M. abscessus*, 1; *M. kansasii*, 1; *M. intracellulare*, 1; *M. malmoense*, 1; e *Mycobacterium* ssp., 1. **Conclusões:** Os resultados obtidos mostraram a importância do diagnóstico bacteriológico das micobacterioses, pois a identificação das espécies possibilita a introdução de um tratamento adequado precocemente.

Descritores: Micobactérias atípicas/isolamento & purificação; Micobactérias atípicas/classificação; Técnicas de diagnóstico e procedimentos.

Introduction

The *Mycobacterium* genus is composed of the *Mycobacterium tuberculosis* complex and other species known as nontuberculous mycobacteria (NTM). In recent decades, the decrease in the prevalence of tuberculosis in developed countries has resulted in an increase in the proportion of diseases caused by NTM.[1] Among these

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species, the *M. avium complex* (MAC) has emerged as a major human pathogen, being a common cause of disseminated disease and death in patients with HIV/AIDS.[2-5]

Mycobacteria occur naturally in many reservoirs: ground water, drinking water from water supply systems, soil, aerosols, protozoan parasites, animals and human beings.[6-10] There is little evidence of person-to-person transmission, although there are recent reports of contamination of patients due do inadequate cleaning and disinfection of medical equipment.[5,11]

Chronic pulmonary disease is the most common clinical manifestation among the diseases caused by NTM, and the most common pathogens are the species belonging to the MAC, followed by *M. kansasii*. The clinical characteristics of NTM-related pulmonary disease are, in many cases, extremely similar to those of tuberculosis. Other clinical manifestations are caused principally by *M. fortuitum, M. chelonae* and *M. abscessus* due to peritoneal infection as a result of catheterization, postsurgical infections, such as those following mammoplasty and heart transplant, as well as those following invasive procedures such as videoscopy and cosmetic surgery. The pathogen most frequently isolated in the case of disseminated disease is the MAC.[2,9,11-13] In addition, there are reports of an increased incidence of NTM isolation in laboratories worldwide, and advances in molecular techniques have led to the diversification of the species identified.[5,14,15]

Since the treatment and epidemiology of mycobacteriosis differ significantly from those of tuberculosis, the rapid and accurate identification of these organisms is mandatory for making a correct diagnosis and initiating an appropriate treatment regimen.[16]

The *Instituto Adolfo Lutz-Regional de São José do Rio Preto* (IAL-SJRP, Adolfo Lutz Institute, São José do Rio Preto Regional Laboratory) is a referral center for the laboratory diagnosis of tuberculosis within the XV subdivision (Riberão Preto, Brazil) of the *Divisão Regional de Saúde* (DRS-XV, Regional Health Care Division) of the state of São Paulo. It carries out mycobacteria cultures when required by the professionals of the Public Health Care Facilities which participate in the DRS-XV Tuberculosis Control Program.

Our objective was to study the occurrence of NTM and the variability of the species isolated in the region served by the IAL-SJRP between 1996 and 2005, as well as to show the importance of the laboratory diagnosis.

Methods

The following laboratory data were obtained from the IAL-SJRP records between January of 1996 and December of 2005: number of analyzed specimens; isolation site; number of positive cultures; and identification of the species. For each patients, one or more clinical specimens from sterile and nonsterile sites were received. The isolations were obtained from pulmonary specimens (sputum, gastric lavage, bronchial lavage and tracheal secretion), ganglion specimens (lymph nodes and secretion), skin specimens (abscess, secretion, wound), liquid specimens (peritoneal and pleural) and blood specimens. Those specimens were processed according to isolation techniques standardized in the guidelines of the Brazilian National Ministry of Health,[17] and positive cultures were sent to the IAL Central Laboratory for identification of the species through phenotyping[8,18,19] and molecular methods[14,15,20] The strain cultures were submitted to screening using macroscopic analysis (morphology and pigmentation) and microscopic analysis (presence of cord factor). The cultures presenting rough nonphotochromogenic colonies and cord factor were excluded from the sample. The strains were identified through phenotyping methods previously described by other authors,[4] who included the analysis of the time of growth using tests with picric acid, sodium

Table 1 - Frequency of isolation of mycobacteria in the Regional Health Care Division of Ribeirão Preto region between 1996 and 2005.

Year	Positive culture results, n	Strains Mtb, n (%)	Strains NTM, n (%)
1996	118	96 (81.3)	22 (18.7)
1997	175	153 (87.4)	22 (12.6)
1998	157	131 (83.4)	26 (16.6)
1999	134	121 (90.3)	13 (9.7)
2000	192	146 (76.0)	46 (24.0)
2001	90	58 (64.5)	32 (35.5)
2002	181	100 (55.2)	81 (44.8)
2003	147	103 (70.0)	44 (30.0)
2004	50	39 (78.0)	11 (22.0)
2005	56	36 (64.3)	20 (35.7)
Total	1,300	983 (75.6)	317 (24.4)

Mtb: *M. tuberculosis*; NTM: nontuberculous mycobacteria.

nitrite, sodium chloride and common agar, as well as growth temperature (26°C, 37°C and 45°C), photochromogenic test, biochemical tests (arylsulfatase—3 and 15 days; β-galactosidase; nitrate reductase, catalase and urease activity; use of sugars; and Tween 80 hydrolysis test) and the test of growth in the presence of the p-nitrobenzoic acid. The molecular identification was carried out, concomitantly with the phenotypic identification, using the polymerase chain reaction and restriction enzyme analysis (PRA) method, which we had modified in a previous study,[21] based on the amplification of a 441-bp fragment of the hsp65 gene and on the analysis of the restriction profiles generated by the enzymes BstEII and HaeIII, separately.

This study is part of the "Study of the diversity in molecular methods of identification of mycobacteria in public laboratories in the State of São Paulo", approved by the IAL Ethics Committee in 2001 (CTC-BM 31/2001).

Results

Over a 10-year period in the DRS-XV region, 1,300 cultures of mycobacteria were isolated from clinical specimens analyzed in the IAL-SJRP, of which 983 (75.6%) were identified as *M. tuberculosis* and 317 (24.4%) were identified as NTM (Table 1) The distribution of the 317 isolations of NTM, from 216 patients, was heterogeneous in this period, the index being highest in 2002—81 strains (44.8%)—and lowest in 1999—13 strains (9.7%).

Table 2 shows the frequency of the species of NTM isolated, of which MAC was the most frequently found, with 182 strains (57.4%); followed by *M. gordonae*, with 33 (10.4%); *M. fortuitum*, with 25 (7.9%); *M. chelonae*, with 8 (2.5%); *M. terrae* complex, with 8 (2.5%); and *M. kansasii*, with 7 (2.2%). Thirty-three strains (10.4%) of other species of lower occurrence were identified; 20 strains (6.3%) with identification based only on time of growth

Table 2 - Annual distribution of the frequency of nontuberculous mycobacteria species, by number of specimens isolated at the Adolfo Lutz Institute, São José do Rio Preto Regional Laboratory, between 1996 and 2005.

Species	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Total. n (%)
MAC	20	17	20	11	30	17	41	16	4	6	182 (57.4)
Mycobacterium gordonae	1	2	2	-	2	1	13	9	1	2	33 (10.4)
M. fortuitum	-	-	2	-	5	5	7	4	-	2	25 (7.9)
M. chelonae	-	-	2	2	2	1	-	1	-	-	8 (2.5)
M. terrae complex	-	-	-	-	1	2	5	-	-	-	8 (2.5)
M. kansasii	1	3	-	-	1	-	2	-	-	-	7 (2.2)
M. abscessus	-	-	-	-	-	-	3	1	2	-	6 (1.9)
M. intracellulare	-	-	-	-	-	-	-	1	1	4	6 (1.9)
M. scrofulaceum	-	-	-	-	-	-	1	1	-	1	3 (0.9)
M. peregrinum	-	-	-	-	-	-	3	-	-	-	3 (0.9)
M. shimoidei	-	-	-	-	-	1	1	-	-	-	2 (0.6)
M. malmoense	-	-	-	-	-	2	-	-	-	-	2 (0.6)
M. neoaurum	-	-	-	-	-	-	-	-	1	-	1 (0.6)
M. flavescens	-	-	-	-	-	-	-	-	1	-	1 (0.3)
M. interjectum	-	-	-	-	-	-	-	1	-	-	1 (0.3)
Mycobacterium sp.	-	-	-	-	2	-	-	6	1	-	9 (2.8)
SGNP	-	-	-	-	2	1	2	-	-	3	8 (2.5)
RGNP	-	-	-	-	1	-	3	1		1	6 (1.9)
SGS	-	-	-	-	-	-	-	3	-	1	4 (1.3)
RGP	-	-	-	-	-	1	-	-	-	-	1 (0.3)
RGS	-	-	-	-	-	-	-	-	-	-	1 (0.3)
Total, n (%)	22 (7.0)	22 (7.0)	26 (8.2)	13 (4.1)	46 (14.5)	32 (10.0)	81 (25.5)	44 (13.9)	11 (3.5)	20 (6.3)	317 (100)

MAC: M. avium complex; SGNP: slow-growing nonphotochromogenic (mycobacterium); RGNP: rapid-growing nonphotochromogenic (mycobacterium); SGS: slow-growing scotochromogenic (mycobacterium) RGP: rapid-growing photochromogenic (mycobacterium); and RGS: rapid-growing scotochromogenic (mycobacterium).

and pigmentation; and 9 (2.8%) had no conclusive identification of the species, and only the genus was identified. In the studied period, the cause of the disease was MAC in 56 patients, *M. fortuitum* in 6, *M. gordonae* in 3, *M. chelonae* in 2, *M. abscessus* in 1, *M. kansasii* in 1, *M. intracellulare* in 1, *M. malmoense* in 1 and rapid-growing mycobacteria in 1 (Table 3). Overall, 72 patients were characterized as cases of mycobacteriosis, according to the bacteriological criteria established by the American Thoracic Society in 2007.

Pulmonary sites had the greatest occurrence of MAC in this period (78.6%). Among the specimens collected from these sites, MAC strains were most common in the sputum specimens, with 132 strains (73.2%), compared with 11 strains (6%) isolated from gastric and bronchial lavages. Among the specimens collected from the sterile sites, MAC strains were most common in blood specimens, with 28 isolations (15.4%).

Table 4 presents the frequency of patients with isolation of NTM from sterile sites. The most frequently isolated species was MAC, from blood specimens, characterizing disseminated disease in 24 patients (58.5%).

Discussion

The number of known species belonging to the *Mycobacterium* genus has doubled in the last 15 years, with the identification of many new clinically significant species.[10,22] Although many studies suggest that the incidence of mycobacteriosis has increased in recent decades, since the reporting of these diseases is not mandatory in Brazil, this observation cannot be proven without official records.

The occurrence of NTM (24.4%) in the studied region, in the 10-year period evaluated, can be compared with that reported in studies conducted at the Clementino Fraga Filho Hospital University

Table 3 - Characteristics of the nontuberculous mycobacteria species isolated between 1996 and 2005.

Species	Number of specimens		Number of patients		
	Total	Respiratory	Total	Total cases	Pulmonary
	n (%)	n (%)	n (%)	n (%)	n (%)
MAC	182 (57.4)	143 (53.3)	106 (49.1)	56 (77.8)	21 (60.0)
Mycobacterium gordonae	33 (10.4)	33 (12.2)	27 (12.5)	3 (4.1)	3 (8.5)
M. fortuitum	25 (7.9)	22 (8.1)	24 (11.1)	6 (8.3)	4 (11.4)
M chelonae	8 (2.5)	7 (2.6)	6 (2.8)	2 (2.8)	2 (5.7)
M. terrae complex	8 (2.5)	8 (2.9)	8 (3.7)	0	0
M. kansasii	7 (2.2)	6 (2.2)	4 (1.8)	1 (1.4)	1 (2.9)
M abscessus	6 (1.9)	6 (2.2)	4 (1.8)	1 (1.4)	1 (2.9)
M. intracellulare	6 (1.9)	6 (2.2)	5 (2.3)	1 (1.4)	1 (2.9)
M. scrofulaceum	3 (0.9)	3 (1.1)	2 (0.9)	0	0
M. peregrinum	3 (0.9)	2 (0.7)	3 (1.4)	0	0
M. shimoidei	2 (0.6)	2 (0.7)	1 (0.5)	0	0
M. malmoense	2 (0.6)	2 (0.7)	2 (0.9)	1 (1.4)	1 (2.9)
M. neoaurum	1 (0.6)	1 (0.3)	1 (0.5)	0	0
M. flavescens	1 (0.3)	1 (0.3)	1 (0.5)	0	0
M. interjectum	1 (0.3)	1 (0.3)	1 (0.5)	0	0
Mycobacterium sp.	9 (2.8)	9 (3.3)	6 (2.8)	0	0
SGNP	8 (2.5)	7 (2.6)	4 (1.8)	0	0
RGNP	6 (1.9)	6 (2.2)	5 (2.3)	1 (1.4)	1 (2.9)
SGS	4 (1.3)	4 (1.5)	4 (1.8)	0	0
RGP	1 (0.3)	1 (0.3)	1 (0.5)	0	0
RGS	1 (0.3)	1 (0.3)	1 (0.5)	0	0
Total	317 (100)	271 (100)	216 (100)	72 (100)	35 (100)

MAC: *M. avium* complex; SGNP: slow-growing nonphotochromogenic (mycobacterium); RGNP: rapid-growing nonphotochromogenic (mycobacterium); SGS: slow-growing scotochromogenic (mycobacterium); RGP: rapid-growing photochromogenic (mycobacterium); and RGS: rapid-growing scotochromogenic (mycobacterium).

Table 4 - Frequency of patients with nontuberculous mycobacteria isolated in specimens from sterile sites.

Site	Identified species		Total, n (%)
	MAC	*Mycobacterium fortuitum*	
Blood	24	1	25 (78.1)
Ganglion/ganglion secretion	5	1	6 (18.8)
Peritoneal fluid	-	1	1 (3.1)
Total	29	3	32 (100)

MAC: *M. avium* complex.

(Rio de Janeiro, Brazil): from August of 1996 to July of 1997, the occurrence of NTM was 15%; and from 2000 to 2002, the occurrence was 45%.[23,24] During the same periods, the occurrence of NTM isolation at the IAL-SJRP was 15% and 34.3%, respectively. In Brazil, it is still difficult to compare data related to NTM, since the few existing studies are isolated, and there are variations in terms of study period, type of clinical specimens and population analyzed.[4] The increase in the frequency of NTM since 2001 can be explained by the implementation of the automated method with liquid culture, a method which is more sensitive than is traditional culture. The decrease in the demand for cultures since 2004 occurred due to a partial interruption in the identification service of the IAL Central Laboratory and can also be attributed to the improvement of the patients treated at the centers specializing in STD/AIDS, with the introduction of the highly active antiretroviral therapy (HAART) in the region. Despite the decrease in the number of strains analyzed in the laboratory from 2003, the percentages show that there was an increase in NTM isolation.

In the present study, the identification of the species at the IAL Central Laboratory was conducted using phenotyping tests until 2000 and using the PRA-hsp65 method thereafter. The species *M. avium* and *M. intracellulare* were differentiated using molecular techniques, whereas, in the previous phase, they had been indentified as MAC. Although the use of the term MAC is taxonomically accepted, it hides the unique epidemiologic characteristics of each species. The complex with greatest occurrence was MAC (57.4%), being 34.4% among the patients with AIDS. After this period, the occurrence of *M. intracellulare* was 1.9%.

The isolation of MAC from the specimens of 55 patients evaluated in the present study allowed the bacteriological classification of the disease according to the bacteriological criteria established by the American Thoracic Society. According to one group of authors,[25] who showed situations in which asymptomatic patients presented sputum smear positivity for MAC and radiologic alteration, the bacteriological data are of great relevance for the characterization of MAC-related pulmonary disease. Hematogenous dissemination of MAC was identified in 24 (43.6%) of these 55 patients.

The second greatest occurrence was *M. gordonae* (10.4%), normally considered saprophytic, which can cause lung and skin infection, principally in immunocompromised patients,[26] although it also shows that there can be transient colonization by NTM. *M. fortuitum* (7.9%), the third most frequently found species, is considered potentially pathogenic and has been responsible for many recently reported outbreaks.[11]

Since NTM and humans occupy the same habitats, and since there has been a steady increase in the number of individuals susceptible to infections caused by mycobacteria (e.g., immunocompromised individuals), it is likely that the prevalence of mycobacteria has increased.[27] In addition, new technologies have been developed with the objective of identifying these agents more efficiently and rapidly. The data obtained in the present study confirm these statements, since, prior to the introduction of the molecular method (in the year 2000), only the species listed in the phenotypic tables were identified. Since 2000, not only has the frequency of the other species increased, but the number of cases due to other NTMs of clinical importance, such as the *M. intracellulare*, *M. abscessus*, *M. chelonae* and *M. malmoense*, has also grown.

Due to the high prevalence of tuberculosis in Brazil, treatment with antituberculosis drugs is initiated immediately after test results indicate an infection due to mycobacteria. The treatment of infections caused by NTM varies depending on the species and requires alternative drugs for a long period of time (18 to 24 months). In the state of São Paulo, Brazil, cases must be reported to the Epidemiological Surveillance Center-Tuberculosis Division in order for the National Ministry of Health to provide the necessary drugs.

The present study demonstrated the variability of the species of NTM in the subdivision of the region of São José do Rio Preto and the clinical importance of certain species, whose correct identification was

fundamental in the diagnosis of mycobacteriosis. The establishment of an appropriate treatment regimen for infection with a given species remains difficult, although only the elucidation of these cases enables the correct diagnosis and the establishment of an appropriate treatment strategy.

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