

# Identification of mycobacteria isolated at University Hospital of Santa Maria, Rio Grande do Sul, Brazil

## *Identificação de micobactérias isoladas no Hospital Universitário de Santa Maria, Rio Grande do Sul, Brasil*

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### ABSTRACT

This study evaluated the prevalence of nontuberculous mycobacterium (NTM) in relation to the total number of cases of mycobacterial infections detected in patients admitted at the University Hospital of Santa Maria from 2008 to 2010. From the positive samples for the genus *Mycobacterium*, 67% belonged to the *Mycobacterium tuberculosis* complex (MTBC) and 33% of them were classified as NTM. This investigation aims to contribute to the epidemiology of mycobacterioses, inasmuch as patients infected by NTM require distinctive treatment and monitoring in comparison with those infected by MTBC.

**Key words:** nontuberculous mycobacteria; *Mycobacterium tuberculosis*; classification; phenotyping; South of Brazil.

### INTRODUCTION

The genus *Mycobacterium* comprises species from the mycobacterium tuberculosis complex (MTBC) and other species denominated nontuberculous mycobacterium (NTM)<sup>(8)</sup>. The protocols for tuberculosis treatment (TB) and infections caused by NTM differ from each other. Thus, the differential diagnosis of tuberculosis and NTM is crucial in order to establish a suitable treatment<sup>(5, 15)</sup>.

In countries with low or average income levels, in which over 90% of the cases of TB occur, the diagnosis is reached through acid-alcohol resistant bacilli smear<sup>(12)</sup>. To date, the diagnosis that confirms tuberculosis is conducted through culture of lesion sample with isolation and identification of the bacilli<sup>(2)</sup>. Several laboratories do not perform this identification procedure due to the lack of resources to apply molecular techniques or the considerable time allocated for phenotypic methods. This study is based on patients admitted at the University Hospital of Santa Maria (UHSM), which currently does not conduct the differential diagnosis of mycobacterioses as standard procedure, but exclusively in cases of treatment failure. Therefore, the objective of the present research was to assess the prevalence of NTM in relation to the total number of cases of identified mycobacterium in patients admitted at the UHSM from 2008 to 2010.

### METHODS

This study was conducted with positive samples for mycobacterium detected through AARB analysis and originally from the clinical analysis laboratory of the University Hospital of Santa Maria (UHSM), located in Santa Maria, RS, Brazil, from January 2008 to December 2010. The information on the positive samples included gender, isolated site and results from sputum smear and culture. Both procedures were routinely applied by the CAL-UHSM and standardized according to the guidelines described in the Bacteriology Manual of Tuberculosis proposed by the Ministry of Health<sup>(4)</sup>.

171 pulmonary and extra-pulmonary positive samples for mycobacterium were categorized by phenotypic methods. The classification of NTM and MTBC was carried out at the Mycobacteriology laboratory of the Clinical and Toxicological Analysis Department of UFSM in order to assess growth time, pigment production and growth in the presence of inhibitor agent<sup>(4)</sup>. The Certificate for Ethical Appreciation (*Certificado de Apresentação para Apreciação ética* [CAAE]) number 0104.0.243.000-11 was issued and the research project was submitted to the Ethics Committee in Research from UFSM.

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## RESULTS

The mycobacterium were classified into four groups:

- I – photochromogenic;
- II – scotochromogenic;
- III – non-chromogenic;
- IV – rapid growth.

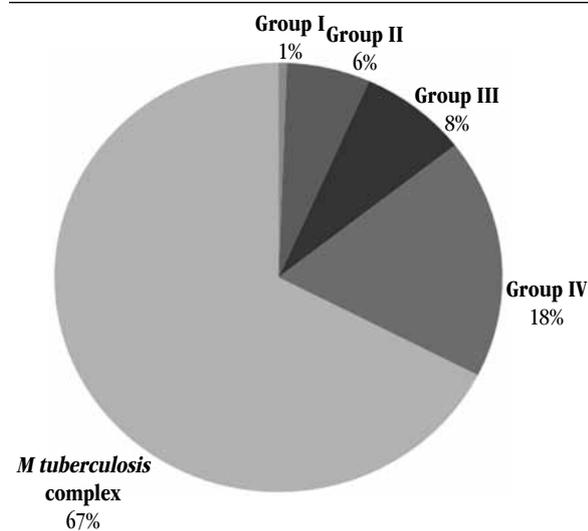
The specimens identified as group III were isolated from *M. tuberculosis* complex through susceptibility to para-aminobenzoic acid (PABA).

4,603 samples were analyzed herein, from which 194 were positive for smear and culture. From the positive samples for *Mycobacterium*, 67% were from *M. tuberculosis* complex and 33% of them were classified as NTM, as shown in **Figure 1**.

70% of 194 samples of isolated bacterium were from male patients, from which 67% were from MTBC and 33% were NTM. In NTM isolates, 70% were from male patients. In **Figure 2**, the occurrence of MTBC and NTM is demonstrated in relation to the isolation sites of positive samples.

## DISCUSSION

The clinically significant species of the genus *Mycobacterium* have doubled in the last 15 years<sup>(9)</sup>. As mycobacterioses are not mandatorily notified in Brazil, the high incidence of these diseases reported in some studies is not corroborated by official records<sup>(3)</sup>. The investigations in this area are scarce and the available ones differ as to the analyzed population and type of clinical sample, hence the difficulty in comparing yielded results<sup>(16)</sup>.



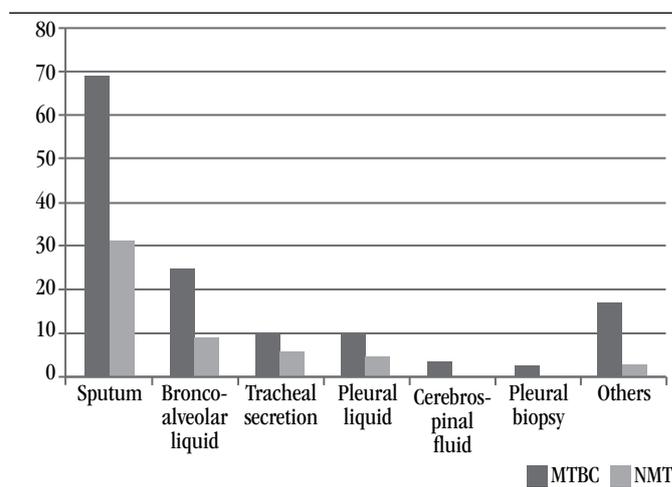
**FIGURE 1** – Percentages of mycobacterium classification isolated from 2008 to 2010

Group I: photochromogenic; group II: scotochromogenic; group III: non-chromogenic; group IV: rapid growth.

From the studies that revealed the percentage of NTM, those conducted in Rio de Janeiro<sup>(7,14)</sup> yielded results similar to the present investigation (33%), with 45% and 34.3% of NTM<sup>(1)</sup>. However, they were carried out from 2000 to 2002. A more recent study was conducted in São José do Rio Preto-SP from 1996 to 2005<sup>(13)</sup>, which showed the incidence of 24.4% of NTM, corroborating our results.

According to Matos *et al.*<sup>(10)</sup>, a study developed in Bahia-Brazil evinced a higher prevalence of NTM among male patients (68.4%), which was also confirmed herein (70%). However, the percentage of NTM isolated from 1998 to 2003 was 8.2%, contrasting with our study (33%). This discrepancy may have occurred owing to the fact that more accurate procedures for the differentiation of colonies infected by NTM were applied in this investigation such as the use of criteria proposed by the American Thoracic Society (ATS)<sup>(6)</sup>. The fact that cases of contamination or colonization were not assessed may have contributed to the overestimation of prevalence values, hence the importance of evaluating both laboratory and clinical findings. The different incidences surveyed herein, including the research group, may have arisen due to the fact that the study was carried out with respiratory and other site samples, which had not occurred in all cited studies. Another plausible explanation is the geographic distribution of NMT species in such a large country as Brazil with several environmental factors<sup>(1, 11)</sup>, which also justifies the age differences in the studied population. Older individuals are prone to preexisting pulmonary diseases, which is a major factor in colonization and/or NMT infection.

Studies solely based on NMT classification (Figure 1) without the identification of the corresponding species were not found, which hindered comparisons. Notwithstanding, as the objective of the present investigation was to raise awareness about the existence of NMT in the region of Santa Maria, we believe this goal was achieved as demonstrated by the yielded results. There were no differences between the type of sample in which MTBC bacilli and NMT were isolated, although there was a higher occurrence in the sputum samples. This result may account for the fact that this kind of sample



**FIGURE 2** – Distribution of mycobacterium as to isolated sites  
MTBC: Mycobacterium tuberculosis complex; NMT: nontuberculous mycobacterium.

is common among medical investigations, inasmuch as it is not an invasive procedure.

In spite of the study limitations, we may conclude that the prevalence of NMT at UHSM is high and may be responsible for the

treatment failure among some patients with only MTBC. This study contributes to the epidemiology of mycobacterioses, insofar as patients infected by NMT require distinctive treatment and monitoring in comparison with those infected by MTBC.

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## RESUMO

*Foi avaliada a prevalência de micobactérias não tuberculosas (MNT) em relação ao total de casos de micobacterioses identificadas em pacientes do Hospital Universitário de Santa Maria, entre os anos de 2008 e 2010. Entre as amostras positivas para o gênero Mycobacterium, 67% eram do complexo Mycobacterium tuberculosis (CMTB) e 33% foram classificadas como MNT. Este estudo procura contribuir com a epidemiologia das micobacterioses, uma vez que os pacientes infectados por MNT necessitam de tratamento e acompanhamento diferenciado dos infectados pelo CMTB.*

*Unitermos: micobactéria não tuberculosa; Mycobacterium tuberculosis; classificação; fenotipagem; Sul do Brasil.*

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