

# 6.<sup>as</sup> JORNADAS CIENTÍFICAS do IHMT

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**Título: Molecular study of the interaction Nontuberculous mycobacteria-host**

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## Introduction

Nontuberculous mycobacteria (NTMs) are a group of acid-fast bacteria with a large number of environmental microorganisms. Nowadays, the clinical relevance of NTM is increasing due to their opportunistic behavior and the association with specific lung and dermatologic diseases. However, the pathogenicity and virulence factors are still poorly understood.

## Objectives

The goal of this project presentation will be to enlighten the molecular mechanisms that regulate MNTs pulmonary infections. This work includes MNTs that persist in latency or multiply in human pulmonary macrophages.

## Methods

**Bacteria:** A total of 5 NTM strains including 3 reference strains (*M. fortuitum* ATCC6841, *M. avium* ATCC25291, *M. smegmatis* ATCC700084) and 2 clinical isolates from Ricardo Jorge mycobacterial collection (*M. fortuitum* 747/08, *M. avium* 60/08) were used. Another strain from INSA collection, *M. tuberculosis* 70/08, was used as a control.

**Intracellular persistence:** THP-1 differentiated with PMA was used as model of human alveolar macrophages.<sup>1</sup> The intracellular persistence of the NTM strains was followed during 1 or 7 days by CFU enumeration for rapid (RGM) and slow growing mycobacteria (SGM), respectively.

**NTMs complete genome sequencing:** DNA extraction was performed using phenol chloroform method.<sup>2</sup> Full genome sequence was performed using NGS platform MiSeq (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. RAST ([www.rast.nmpdr.org](http://www.rast.nmpdr.org)) and MAUVE platforms were used for annotation and multiple alignments, respectively.<sup>3</sup>

## Results

NTM exhibited different intracellular persistence patterns. Clinical strains were able to replicate whereas reference strains persist in a steady state (*M. fortuitum*) or are cleared by macrophages (*M. smegmatis*). In order to explore a possible connection between these results and NTM genes an all genome analysis was adopted. The preliminary data analysis shows that RGM (*M. fortuitum*, *M. smegmatis*) genomes have an average of 6.7 million bp and SGM (*M. avium* and *M. tuberculosis*) an average of 4.8 million bp. For 747/08 and 60/08, the 2 NTM able to replicate within macrophages, the data analysis revealed an increased number of genes and gene copies in specific gene clusters such as fatty acid metabolism and cell envelope gene clusters.

## Conclusions

The preliminary results revealed some suspect gene clusters that might be involved in the intracellular persistence or virulence. Further studies are needed to confirm the role played by these genes in the NTM pathogenesis.

## References

1. Sousa S *et al.* *Int J Mycobacteriology*. 2015;4(1):36-43.
2. Benjak A *et al.* *Methods Mol Biol*. 2015;1285:1-16.
3. Darling ACE *et al.* *Genome Res*. 2004;14(7):1394-1403.