# MYCOBACTERIA OF CLINICAL SIGNIFICANCE IN BRAZIL: MOLECULAR CHARACTERIZATION, INTERACTION WITH THE ENVIRONMENT AND WITH MACROPHAGES

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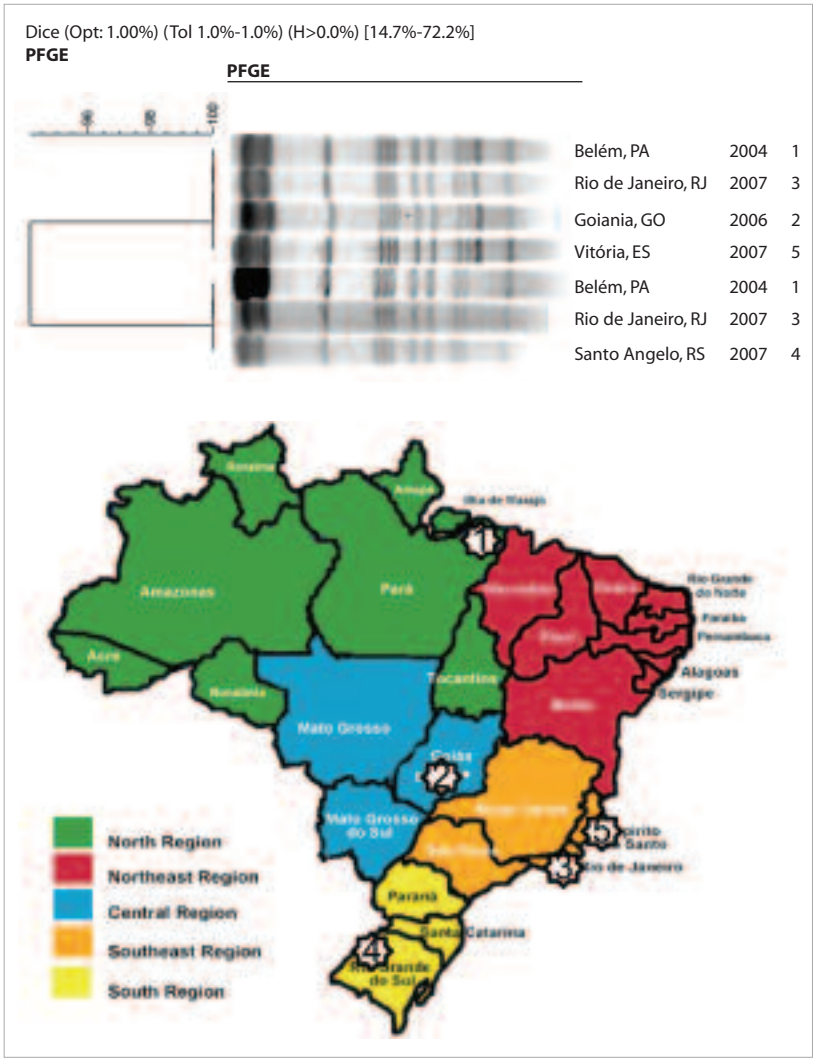
Non-tuberculous micobacteria (NTM) are emerging pathogens, with potential to cause infections in immunocompromized and also immunocompetent patients, frequently as a complication of invasive procedures, like ophthalmologic, plastic, and laparoscopic surgeries, cosmetic procedures, and endoscopically based exams. Studies that improve our knowledge about the biology of these bacteria and the interaction with the environment and host cells will be useful for future elaboration of control programs and therapeutic approaches. In sub-project 1, three molecular methods will be used to type isolates from an outbreak of infections after surgical procedures that occurred at Belém (PA) between 2004 and 2005. The results will be compared with patients' data for analysis of epidemiological correlation. A bank of genetic profiles will be organized and a fluxogram will be elaborated to help in the management of future outbreaks. In sub-project 2, we will investigate the presence of plasmids and mycobacteriophages in outbreak and environment isolates and their role in horizontal transfer of genetic information. In sub-project 3, the potential of these bacteria to produce and to survive in biofilms will be evaluated and the role of these structures in resistance to biocides commonly used for equipment disinfection will be investigated. In sub-project 4, the interaction of a *M. avium* virulent isolate with epithelioid-like cells (recombinant interleucine-4 treated murine peritoneal macrophages), constituting an *in vitro* model developed by our group, will be investigated to improve our understanding of the functions of epithelioid cells present in granuloma.




*PFGE patterns of representative isolates from each outbreak (Belém, Goiânia, Rio de Janeiro, Santo Ângelo, and Vitória). Two PFGE patterns, * and **, differing in one band, were identified in outbreak isolates. Columns indicate the city, year of the outbreak, and map location, respectively.*

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We are presently involved in collaborative projects to extend the molecular studies and also to determine the susceptibility to antimicrobial agents of a significant subset of *Mycobacterium massiliense* isolates. An outbreak of infections affecting 311 patients submitted to different invasive procedures occurred in 2004-2005, at the city of Belém, in the North region of Brazil. In 2007, similar cases were detected in other States from Brazil – Goiás, Rio de Janeiro, Rio Grande do Sul, Espírito Santo and Paraná. A subset of isolates from these sites was compared to the Belém isolates and results of DNA sequencing and PFGE confirmed, unexpectedly, that the same *M. massiliense* strain was responsible for the outbreaks in the different States.

In a previous project we have detected by PCR the presence of a *Mycobacterium avium* specific insertion sequence, IS1245, in colonies of *Mycobacterium kansasii*. Both species were isolated from a bone marrow sample from the same HIV-positive patient. The presence of this element in isolated colonies of *M. kansasii* was confirmed by Restriction Fragment Length Polymorphism (RFLP) using a probe complementary to the IS1245. A plasmid was detected by PFGE in colonies of *M. avium* and *M. kansasii* which generate amplicons by PCR-IS1245 while colonies of *M. kansasii* that did not generate amplicons by PCR-IS1245 did not bear this plasmid. These results opened new perspectives for this project, and we are engaged in the characterization of this plasmid and the study of conjugative mechanisms in mycobacteria. There are few studies about horizontal gene transfer in mycobacteria and results obtained with this project will boost up the understanding of mechanisms of gene acquisition and its influence in virulence, evolution and genetic diversity in mycobacteria.

Our group succeeded in the obtention of an *in vitro* model treatment, during 7 days, of mouse resident peritoneal macrophages with r-IL-4) for the generation of epithelioid-like cells (ECs-like) that can be used as a surrogate of epithelioid cells (ECs) found in granulomas. We are presently studying cell signaling in mouse peritoneal macrophages upon rIL-4 treatment, before the performance of experiments with infected cells. Experiments to obtain *M. avium* expressing green fluorescent protein are under way and the recombinant bacteria will be used in trafficking experiments.

Rough and smooth variants from one isolate from the Belém outbreak were separated and these phenotypes were shown to be stable after ten passages in solid medium. Histopathological analyses of the excised spleens are under way. *Ex vivo* experiments to study the infection of ECs-like and macrophages with the two variants will be also performed to check how these cells cope with the infection. The results will be compared to those obtained with environmental isolates of the same species.

## MAIN PUBLICATIONS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