

ANTS AS MECHANICAL VECTORS OF MYCOBACTERIA IN A HOSPITAL: PRELIMINARY DATA

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Abstract To evaluate the potential of ants as vectors of mycobacteria in a hospital specialized in the care of patients with tuberculosis of the São Paulo State, Brazil, ants were collected from different areas between 2008 and 2010. Procedures for isolation and identification of mycobacteria were performed, and the material was inoculated into specific culture media. The suggestive cultures were subjected to Ziehl-Neelsen stain for acid-fast bacilli and identification was performed by molecular methods (PRA with the gender-specific pair of primers TB11 - TB12 and genetic sequencing). Preliminary data shows that of 210 processed samples, seven fast-growing mycobacteria were isolated (2 *Mycobacterium chelonae*, 1 *Mycobacterium chubuense*, 1 *Mycobacterium murale* and 3 not identified yet). *M. chelonae* was previously reported as causative agent of abscess in humans. These data confer that ants can carry mycobacteria, acting as mechanical vectors and a factor of potential risk of infection for patients and health professionals.

Key Words Ants, tuberculosis, hospital, mycobacteria, vector mechanical

INTRODUCTION

The processes of urbanization trigger numerous disorders including: growth and disorderly exacerbated the population, water contamination, and difficulty of sanitary control, resulting in the dissemination of arthropods and diseases, since these animals are responsible for the decrease in quality of human life and a growing public health problem. (Bueno and Campos-Farinha, 1998; Campos-Farinha et al., 2002; Oliveira and Campos-Farinha, 2005; Freitas and Teixeira, 2007; Pesquero et al., 2008).

Ants are among the arthropods best adapted and benefited by the human community, and with greater diversity in the tropics. The global estimates is about 18,000 species of ants, and of these 2.000 have been cataloged in Brazil, less than 1% could be considered urban pests because of the close association with man (Bueno and Campos-Farinha, 1999; Campos-Farinha, 2002; Oliveira and Campos-Farinha, 2005; Santos et al., 2009). The presence of ants in a hospital carries a more serious risk to public health, especially for its ability to carry pathogenic microorganisms, acting as mechanical vectors, enabling their involvement in nosocomial infections (Bueno and Campos-Farinha, 1999; Santos et. al., 2002; Santos et. al., 2009; Tanaka et. al., 2007).

Nosocomial or hospital infections represent a major concern and have attracted great interest in science, to promote high rates of morbidity and mortality in hospitalized patients. According to the indexes infections are between 3.8 and 10% in developed countries, which could increase about 50% in developing countries (Maia et. al., 2009; Pesquero et al., 2008; Tanaka et al., 2007).

Research conducted have shown the importance of ants as vectors of pathogens, including species of mycobacteria relevant to Public Health, as *Mycobacterium tuberculosis* (Roxo et al., 2010). The objective of this research is to evaluate the potential of ants as mechanical vectors of mycobacteria in a hospital specialized in treating patients with tuberculosis.

MATERIAL AND METHODS

The hospital was selected is part of the Secretary of Health of São Paulo and specializes in caring for patients with tuberculosis and internal social ill with complications from tuberculosis or multi-drug-resistant. This made conditions favorable for the presence of ants, detected in previous visit before the site selection.

Samples. The ants were collected in the hospital during the period from 2008 to 2010, using polypropylene cryovials, sterile, with screw cap. Ant collections were made by active search with manual collection and use of baits distributed in different facilities of the hospital where the ants have been not previously detected. Two samples of ants were performed in the same facility. One of them for laboratory procedures with the aim at evaluating the presence of mycobacteria and the other for ant identification.

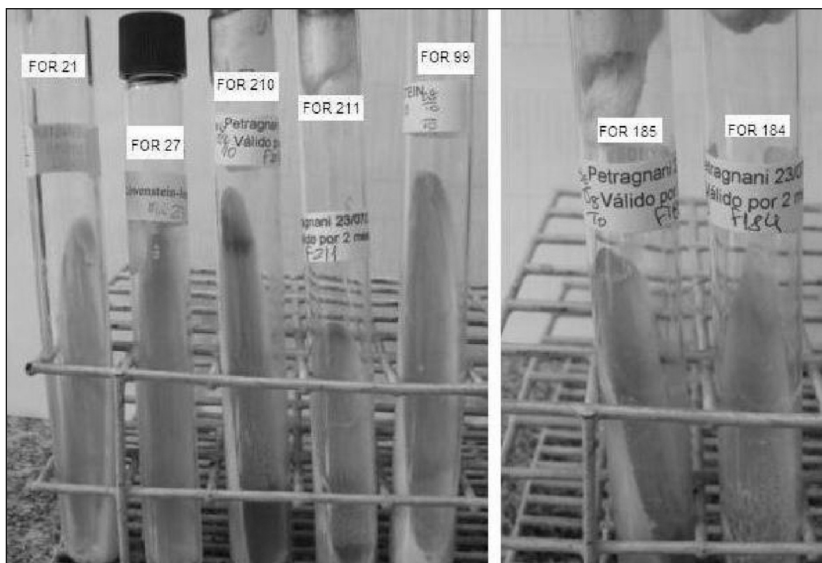
Isolation of mycobacteria. For the isolation of mycobacteria (Brasil, 2005) performed at the Laboratory of Tuberculosis Animal of the Center for Animal Health to Instituto Biológico of São Paulo, the samples were initially frozen at -18°C for at least 24 hours, macerated and suspended in sterile saline and inoculated in Löwenstein-Jensen culture (LJ) which allows the growth of mycobacteria of medical interest and Stonebrink employed for the isolation of *Mycobacterium bovis* and incubated at 37°C for 90 days. The suggestive cultures were subjected to Ziehl-Neelsen stain for acid-fast bacilli and identification was performed by molecular methods.

Identification of mycobacteria. For the identification of species molecular methods were used as the Polymerase Chain Reaction (PCR) for the detection of species belonging to *M. tuberculosis* Complex, PCR with restriction analysis – PRA for the identification of environmental mycobacteria by using the primers Tb11 (5'-ACCAACGATGGTGTGCCAT) and Tb12 (5'-CTTGTCGAACCGCATACCCT) and digestion by *BstEII* and *HaeIII* enzymes. The amplification and digestion of fragments occurred as described by Telenti and colleagues (1993), and compared to PRASITE database.

The methodology of DNA sequencing was also used to confirm the mycobacteria species identification, and this procedure has yet in progress for some isolates. Partial hsp65 gene amplification was performed with primers Tb11 and Tb12 and rpoB gene fragment with the primers MycoF (5'-GGCAAGGTCACCCCGAAGGG-3') and MycoR (5'-AGCGGCTGCTGGGTGATC-3'). PCR products were purified using the Illustra GFX DNA and Gel Band Purification kit (GE Healthcare, Buckinghamshire, UK). The sequences of hsp65 and rpoB genes were obtained with ABI3130 sequencer and Big Dye Terminatorv3.1 Cycle sequencing kits (Life Technologies, Carlsbad, Ca) using standard protocols and same primers used for amplification separately.

RESULTS

Two hundred and ten samples were processed, to date. Seven of them were isolated as rapidly growing mycobacteria. Characterized by molecular methods (PRA and sequencing) as two of the *Mycobacteria chelonae*, one of *Mycobacteria chubuense*, one of *Mycobacteria murale*, and three as even unidentified species of *Mycobacterium*.



Cultures of mycobacteria isolated of ants from hospital specialized in treatment of patients with tuberculosis.

DISCUSSION

Preliminary data from our study revealed the importance of ants as potential mechanical vectors of mycobacteria, and the contribution of the same agent spread by different environments in a unique hospital. The isolation of potential pathogenic environmental mycobacterium species carried by ants emphasizes the importance of these individuals as vehicles for disseminating pathogens, causing a great concern for public health professionals, because they are an aggravating factor that supports the expansion of hospital infections making necessary the adoption of measures for controlling arthropods in these places.

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