# *Mycobacterium simiae* Infection in a Patient with Acquired Immunodeficiency Syndrome

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*Mycobacterium simiae* **is usually an environmental contaminant rarely associated with human disease. We report a fatal case of** *M.simiae* **infection in a 37 year old, HIV positive, male from whom the organism was isolated from blood culture. The identification of M.simiae was performed using DNA amplification followed by analysis on 3% agarose gel of the amplicon fragments after digestion by restriction endonucleases. The precise identification of mycobacterial isolates to the species level is important, with both epidemiological and therapeutic implications.**
**Key Words:** *Mycobacterium simiae*, **AIDS, RFLP.**

Since most laboratories in Brazil do not identify *Mycobacterium* other than tuberculosis (MOTT) to the species level, and considering that the most frequent MOTT is *Mycobacterium avium*-intracellulare complex (MAI complex), patients are treated empirically for this group of agents when there is a laboratory report of MOTT.

The number of different organisms included in the category MOTT, is growing, and commercially available products to detect yheir diversity do not fill the Mycobacteria laboratory need for species identification.

The only description of *M.simiae* isolated in Brazil was reported in 1995, in Araraquara, SP [1]. It was isolated from sputum of an HIV positive patient and there was no clinical history and no report of outcome. To our knowledge, this is the first report of *M.simiae* isolated from blood in an AIDS patient in Brazil. The intent of this report is to show the need to identify *Mycobacterium* to the species level in all clinical isolates. Such identification will have both epidemiological and treatment implications.

## Case Report

A 37-year-old man was found to be HIV seropositive 5 years before the diagnosis of *M.simiae* infection. At the first hospital admission, he presented weight loss, diarrhea, and oral candidiasis. At that time, Zidovudine™ and Didanosin™ were initiated. His general condition improved but, due to erratic use of medication, he was admitted to the Infectious Disease Service at Ferreira Machado Hospital, Campos, Rio de Janeiro State, Brazil, in August, 1998, presenting persistent fever, diarrhea, progressive weight loss, and gray skin. At hospital admission, laboratory tests showed: hematrocrit 28%, hemoglobin 9.5 g/100mL, total leukocytes count 2,400/mm$^3$ and CD4$^+$ count 146/ mm$^3$. A bone marrow aspirate was done but the only alteration detected was hypoplasia. Cotrimoxazol™ was prescribed, and the patient's general condition improved. He was discharged using Zidovudine™,

Lamivudine™, Indinavir™, Ketoconazol™ and Cotrimoxazol™. During his outpatient follow-up special blood cultures for *Mycobacterium* sp. and fungi were collected.

The patient was lost to medical care for the next 2 months, but then his general condition worsened. At that time he had significant weight loss, persistent fever, hepatosplenomegaly, diarrhea and a positive blood culture for MOTT. He was admitted to the Infectious Disease Service at Ferreira Machado Hospital on November 18, 1998. Laboratory tests showed: hematocrit 21%, hemoglobin 6.8 g/100mL, total leukocytes count 1,900/mm$^3$, and platelet count 78,000/mm$^3$. Amikacin™, Ethambutol™, Chlaritromycin™ and Ciprofloxacin™ were introduced. After 17 days of hospital stay, he was sent home without fever or diarrhea.

On December 22, 1998, he returned with a recurrence of the signs and symptoms similar to those at last hospital admission. He mentioned that he had used his medication erratically. With regular use of Ciprofloxacin™ (1g/daily), Ethambutol™ (1.2g/daily) and Chlarithromycin™ (1.0 g/daily), his clinical condition improved and he was sent home. One month later his general condition worsened and soon after re-hospitalization, he died.

### Isolation and identification procedures

Five ml of blood were cultivated on each 13A radiometric media and analyzed with Bactec 460TB (Becton-Dickinson). The vial was incubated at 37ºC and read 3 times a week during the first 2 weeks. The vial was positive at the end of the second week. The microscopic examination showed numerous acid fast staining bacteria. A sample of liquid media was planted on Lowenstein-Jensen medium for additional tests. After DNA extraction, a polymerase chain reaction (PCR) was carried out using the set of primers and the protocol described previously by Telenti, et al., [2]. The expected amplicons of 439 base pairs (bp) were visualized on 2% agarose (Life Technologies) gel stained with ethidium bromide (0.05 µg/mL). The amplicons were digested with *Bst*EII and *Hae*III restriction

endonucleases, following the manufacturer´s recommendations (Life Technologies) [2]. The products of *Bst*EII and *Hae*III cleavage were analyzed on 3% Metaphor™ (FMC Bioproducts) agarose gel electrophoresis. *M. simiae* ATCC 29275 and *M. tuberculosis* ATCC 27294 prototype strains were used as control (Figure 1). The strain isolated from the patient showed exactly the same polymorphism restriction analysis (PRA) pattern of *M.simiae* ATCC 29275. The isolate was confirmed as *M. simiae* at the Health Center at Tyler, University of Texas, USA.

### Discussion

*M.simiae* is a photochromogenic environmental acid-fast bacillus that has rarely been associated with human disease. However, pulmonary infections due to this organism have been reported in monkey handlers and in people who have had contact with these primates. It was originally isolated in 1965, from monkeys imported from India to Hungary [3]. Since then, *M.simiae* has been isolated from tap water and soil samples in the Middle East, Central Africa, USA, and Australia [4,5]. In Germany, it has been isolated from water systems from dental units [6].*M.simiae* is a common isolate from clinical specimens in Israel [7] and has been isolated from the water supply in Gaza [8]. In Europe, *M simiae* has been isolated from 28% of stool specimens from 50 volunteers [9]. Reports of *M.simiae* infection in AIDS patients have been previously described [9,10]. The isolate was resistant to all common antituberculous chemotherapeutic agents, as were *M.simiae* from other case reports elsewhere.

Until now, we were not able to find any report on isolation of *M. simiae* from Brazil. In Brazil, the vast majority of mycobacterial isolates are reported as *M. tuberculosis* complex, or MOTT, when a culture is performed. In fact, most primary care public health institutions in Brazil do not perform cultures on patients with clinical diagnosis of tuberculosis. Diagnosis is made only by X-ray and acid fast smear tests from sputum.

Leite, et al. studied 78 mycobacterial isolates from patients with suspected pulmonary tuberculosis during

**Figure 1.** Polymorphism restriction analysis of amplicons of 439bp digested with *Bst*EII and *Hae*III restiction endonucleases.
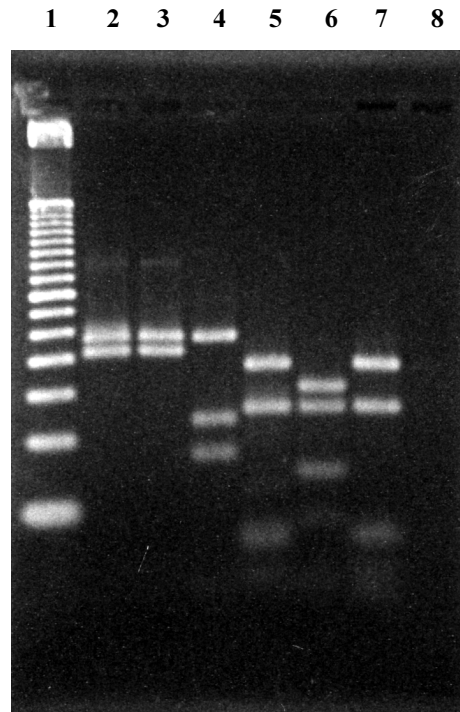


Lane 1: MW 50bp DNA ladder (Life Technologies); Lanes 2-4: *Bst*EII restriction endonuclease digestion of amplicons from *M. simiae* (patient), *M. simiae* ATCC 29275, and *M.tuberculosis* ATCC 27294; Lanes 5-7: *Hae*III restriction endonuclease digestion of amplicons of *M. simiae* (patient), *M. tuberculosis* ATCC 27294, and *M. simiae* ATCC 29275.

the year of 1993, in Araraquara city in São Paulo state. The method used was thin layer chromatography of mycolic acids and some biochemical tests. Among 78 isolates, 69 were *M.tuberculosis*, 5 were *M.avium-intracelullare*, 2 were *M.fortuitum*, 1 was *M.chelonae* and 1 was *M.simiae*. *M.tuberculosis* was the most prevalent among both HIV negative (88.4%) and HIV positive (53.3%) groups. There was only 1 *M.simiae* isolate and it was from HIV positive patient.

It is not always easy to differentiate *M.simiae* from *M.scrofulaceum* based on traditional tests like pigment and niacin production, so chromatography or molecular methods must be used. In our laboratory we are using molecular methods to identify all isolates of *Mycobacterium spp.* From January, 1999, to December, 2000, from a total of 256 mycobacterial isolates, 162 (63.3%) belong to the *M.tuberculosis*

complex, and 94 (36.7%) were non-Tuberculous Mycobacteria (NTM). Among NTM, 2 were identified as *M.simiae*, 1 isolated from blood, and 1 from bronchoalveolar lavage, representing 2.13% of all NTM isolates. Therefore, we believe that *M.simiae* is a present, but under diagnosed, infection.

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