

# IS1245 restriction fragment length polymorphism typing of *Mycobacterium avium* from patients admitted to a reference hospital in Campinas, Brazil

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## Abstract

*Mycobacterium avium* is an important pathogen among immunodeficient patients, especially patients with AIDS. The natural history of this disease is unclear. Several environmental sources have been implicated as the origin of this infection. Polyclonal infection with this species is observed, challenging the understanding of its pathogenesis and treatment. In the present study 45 *M. avium* strains were recovered from 39 patients admitted to a reference hospital between 1996 and 1998. Species identification was performed using a species-specific nucleic acid hybridization test (AccuProbe®) from Gen-Probe®. Strains were genotyped using IS1245 restriction fragment length polymorphism typing. Blood was the main source of the organism. In one patient with disseminated disease, *M. avium* could be recovered more than once from potentially sterile sites. Strains isolated from this patient had different genotypes, indicating that the infection was polyclonal. Four patient clones were characterized in this population, the largest clone being detected in eight patients. This finding points to a common-source transmission of the organism.

## Key words

- *Mycobacterium avium*
- IS1245 RFLP
- Mycobacteria
- AIDS
- Genotyping

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## Introduction

*Mycobacterium avium* has emerged as a frequent pathogen among immunodeficient patients since the advent of AIDS (1). Although antiretroviral treatment has had a significant impact on the course of this disease (2), cases of *M. avium* infection still have a poor prognosis (3-5). The natural history of the disease has not been determined. It seems that the environment itself, including animal reservoirs more than hu-

mans, represents the natural source of bacteria for these patients (6,7). Disseminated disease is observed in AIDS patients, as evidenced by blood, bone marrow and deep tissue cultures (4,8-10). Polyclonal infection has been documented and seems to be frequent (11,12).

Molecular typing techniques are important tools to determine the epidemiology of infectious diseases. Several methods based on amplification, hybridization or restriction can be applied to trace the mechanisms of

disease. DNA fragment restriction and subsequent hybridization with *IS1245* probes have been shown to be suitable for typing *M. avium* infections (13,14).

The AIDS epidemic is a serious health problem. Infections caused by mycobacteria, mainly *M. tuberculosis* and *M. avium*, are frequently seen at our hospital. *M. avium* was identified in 45% of HIV-infected patients with mycobacterial diseases during the year 1996 (8).

The present study was designed to identify and characterize the genetic polymorphism of *M. avium* strains recovered from clinical specimens between 1996 and 1998.

## Material and Methods

### Setting

The study was performed at the Hospital de Clínicas, Universidade Estadual de Cam-

pinas (HC-UNICAMP), Campinas, SP, Brazil. The hospital serves an area with an estimated population of 5 million people. All cultures positive for *M. avium* from 1996 through 1998 were analyzed.

### Isolation and identification of mycobacteria

Primary isolation was performed under safety conditions in the Mycobacterial Laboratory. The isolates were cultivated in 12-B and 13-B bottles of the BACTEC 460TB radiometric culture system (Becton Dickinson® Microbiology Systems, Sparks, MD, USA). Aliquots from positive bottles were transferred to Lowenstein-Jensen (LJ) medium. Mycobacteria were identified on fully grown LJ slants. Species identification was performed with the AccuProbe® *M. avium* complex Culture Identification Test (GenProbe® Incorporated, San Diego, CA, USA).

### Molecular strain typing

*IS1245* restriction fragment length polymorphism (RFLP) was determined according to an internationally standardized protocol (14). Fingerprints were analyzed visually and considered to be identical when all bands shared the same molecular weight positions. *M. avium* reference strain IWGMT49 with fragments of known molecular weights was analyzed together with unknown samples.

## Results

Forty-five *M. avium* isolates were recovered from a variety of clinical specimens from 39 patients (Table 1). Almost all the patients included in the study (N = 35) had documented HIV infection. Forty-three isolates could be typed with the *IS1245* RFLP, while two strains were untypable because of the absence of detectable *IS1245* copies. *M. avium* was recovered from blood, bone marrow, biopsy tissue or cerebrospinal fluid (CSF) in 47% of samples, with blood cul-

Table 1. *Mycobacterium avium* recovered from 39 patients during the 1996-1998 period.

Clinical specimen	Number of isolates (%)
Blood	14 (31.1)
Sputum	12 (26.7)
Stool	5 (11.1)
Urine	4 (8.9)
Liver biopsy	3 (6.7)
Bone marrow	3 (6.7)
Gastric lavage	1 (2.2)
Cerebrospinal fluid	1 (2.2)
Broncho-alveolar lavage	1 (2.2)
Skin	1 (2.2)
Total	45

Table 2. Sources of clinical specimens, number of *IS1245* bands observed, and date of recovery of isolates from a patient with polyclonal and disseminated *Mycobacterium avium* infection.

Source	Number of bands	Date of recovery
Blood	6	2/1/96
Blood	17	9/1/96
Lung biopsy	6	1/3/98

tures being the main source of the organism.

The RFLP patterns of the 43 typable strains consisted of 6 to 23 bands with an average of 17 IS1245 copies per strain. For five patients, more than one isolate could be recovered. In one patient with disseminated disease, two strains with different genotypes were recovered from blood cultures drawn one week apart (Table 2), indicating that he had a polyclonal infection (fingerprints shown in Figure 1). Fingerprinting studies also showed that 16 of 39 patients (41%) could be assigned to four IS1245 clusters. The individual clusters and the distribution of isolates according to the number of IS1245 copies observed are shown in Figure 2. The largest cluster, arbitrarily designated MA18, consisted of eight patients. Some fingerprints of selected isolates belonging to this cluster are shown in Figure 3.

## Discussion

*Mycobacterium avium* infections are frequently detected in AIDS patients in Brazil despite the wide use of highly active antiretroviral chemotherapy (4,9). This infection has been associated with AIDS in the presence of the late immunosuppression that occurs when CD4+ cell counts are extremely low. Antiretroviral treatment has considerably improved the prognosis of AIDS by reducing the incidence of opportunistic events, but treatment failures, and non-adherence to therapy render individuals susceptible to many pathogens. In such patients *M. avium* has been isolated from many clinical sources, including stool, sputum, skin, blood, CSF, and biopsy tissues. Isolation of organisms from these body sites does not necessarily mean that they are a cause of morbidity, unless they are found at sterile sites concomitantly with clinical evidence such as anemia, weight loss and diarrhea (4,9). The epidemiological events involved in the natural history of this infection have not yet been clarified.

Blood was the main source of *M. avium* in our study population, as also reported by others (5,8), and confirms the disseminated character of this infection (4). Oplustil et al. (4) have shown that the *M. avium* complex was recovered from 58.4% of positive vials, corresponding to 53.6% of patients with positive blood cultures.

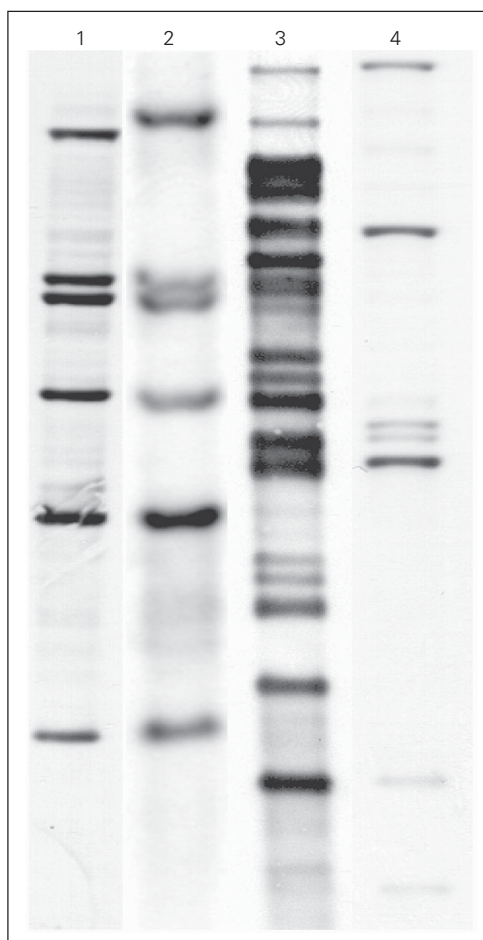


Figure 1. IS1245 fingerprints of *Mycobacterium avium* isolated from a patient with multiple strains recovered. Lanes 1 and 2, Isolates from two blood samples; lane 3, isolate from lung tissue; lane 4, strain IWGMT49.

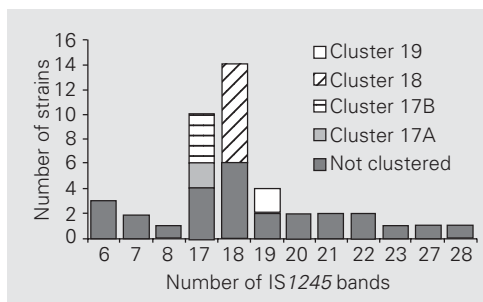
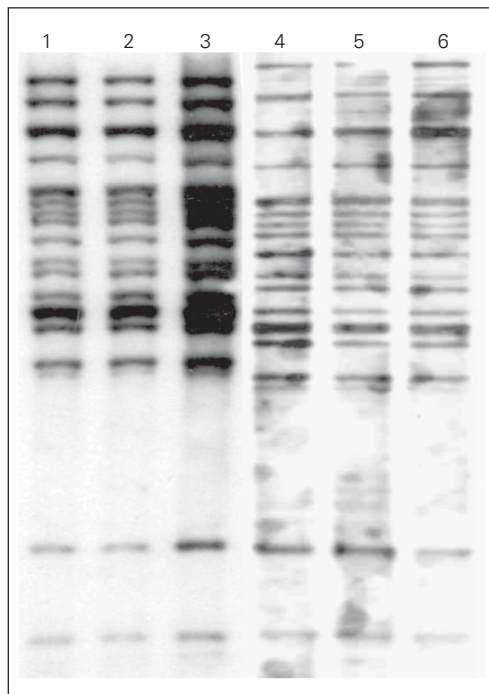


Figure 2. Number of isolates according to the number of IS1245 bands observed. Clustered strains are outlined and represent one strain per patient.

Figure 3. Some fingerprints from the largest cluster found, and arbitrarily designated MA18.



Our study also shows that 41% of the strains recovered from several clinical specimens were in clusters, indicating that these strains were acquired from common sources of infection. However, these sources of infection could not be determined in the present investigation. Since *M. avium* is not trans-

mitted from human to human we suspect that the patients acquired the organism from the hospital environment, probably from water or food. In a recent study (15), it was concluded that *M. avium* complex infection results from diverse and probably undetectable environmental and nosocomial exposure.

Polyclonal infection was demonstrated in one patient in our study. This has been reported previously (12) and it has also been shown that organisms isolated from polyclonal infections demonstrated by organisms with different fingerprints can have different antimicrobial susceptibility (11).

Improved strategies to facilitate identification of HIV-infected people encourage patient enrollment in clinical care, and improve adherence to prevention. However, new treatment regimens will be needed before disseminated *M. avium* complex disease can be effectively prevented.

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