# IS*1245* Genotypic Analysis of *Mycobacterium avium* Isolates from Patients in Brazil

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## ABSTRACT

*Objective:* Disseminated *Mycobacterium avium* infection is an emerging opportunistic disease among patients with acquired immunodeficiency syndrome (AIDS) in Brazil. The mode of transmission of *M. avium* in a developing country setting needs to be better characterized.

*Methods: Mycobacterium avium* strain collections in São Paulo and Rio de Janeiro were analyzed according to the strains' IS*1245* DNA gel electrophoretic migration patterns. Medical records of the patients from whom *M. avium* isolates were available were reviewed, and their demographic characteristics were stratified according to the isolates' IS*1245* DNA fingerprint patterns.

*Results:* Of 105 patients, 33 (31%) with *M. avium* isolated between 1990 and 1994 had strains having IS*1245* patterns identical in patterns seen in isolates from two or more patients (designated as cluster pattern strains). Cluster pattern strains were isolated from 21 (39%) of 54 patients with disseminated infection (defined as infection due to *M. avium* isolated from a sterile site in an adult patient). Six of the cluster pattern strains were isolated only from sterile sites. In São Paulo, cluster pattern strains were significantly more likely to be isolated from patients with disseminated disease.

*Conclusions:* These preliminary observations suggest that in large cities of Brazil, a high proportion (at least 39%) of disseminated *M. avium* infections in patients with AIDS results from a recent transmission. Some strains of *M. avium* may be more likely to cause disseminated disease than others after an infection.

*Mycobacterium avium* is the most common bacterial cause of opportunistic infections in patients with acquired immunodeficiency syndrome (AIDS) in developed countries.[1] However, this opportunistic infection only recently has come to be recognized as an emerging problem in patients with AIDS in middle income countries, such as Brazil.[2] In a recent 2-year study at one hospital for infectious diseases in São Paulo, Brazil, *M. avium* was isolated from bone marrow of 18% of patients with AIDS with persistent fever, anemia, and leukopenia.[2]

Although *M. avium* infection is presumably acquired through an oral route, the sources and mode of transmission of the organism in patients with AIDS remain poorly defined. Subtyping strains by pulsed-field gel electrophoresis (PFGE) has shown that water may be an important reservoir of *M. avium* in some institutional settings in the United States.[3] Serotypic differences in isolates from patients with AIDS in Europe and North America suggest geographic differences in strains associated with disseminated *M. avium* disease.[4] Little evidence for its direct person-to-person transmission exists. In developing countries where the incidence of disseminated *M. avium* infection is increasing, little is known about modes of transmission. Whether disseminated infection represents reactivation of an old infection or recent exogenous infection has not been satisfactorily answered. The recent emergence of *M. avium* infections among human immunodeficiency virus (HIV)-infected persons in Brazil provided an opportunity to characterize the distribution

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of DNA fingerprint patterns of *M. avium* isolates using a strain typing method based on an insertional element called IS*1245*.[5] Such an analysis may provide an opportunity to better define the transmission pattern of this infection in a developing country setting.

## MATERIALS AND METHODS

### Bacterial Isolates and Patients

*Mycobacterium avium* isolates were obtained from a mycobacterial culture collection of Instituto Adolfo Lutz in São Paulo and from two hospital microbiology laboratories and a reference laboratory in Rio de Janeiro. The isolates were recovered between 1990 and 1994 from predominantly HIV-seropositive patients from sterile sites (blood, bone marrow, lymph nodes, and cerebrospinal fluid) and from nonsterile sources (respiratory tract, stool, urine, ear, and skin). *Mycobacterium avium* was identified by standard biochemical tests after the clinical samples were cultured in Lowenstein-Jensen medium at 37°C.[6,7]

Medical records of patients from whom the isolates were recovered were retrospectively reviewed for demographic and clinical information by the clinical staff who cared for the patients. A standardized intake form was used to collect the information. The diagnosis of AIDS in a patient was based on a positive HIV serologic test and presence of one or more AIDS-defining opportunistic infections.

### DNA Fingerprint Analysis

The DNA fingerprint analysis was performed at Cornell University Medical College, according to a recently described method.[5] Among mycobacterial species, the insertional sequence IS*1245* has been reported to be found only among *M. avium* subspecies *avium, sylvaticum,* and *paratuberculosis* (GenBank accession number L33879).[5] The isolates were initially tested for the presence of IS*1245* by amplification by polymerase chain reaction (PCR) of a 427-bp IS*1245* internal sequence. Strains whose DNA failed to be amplified were tested by the *M. avium* and *M. intracellulare* AccuProbe test (GenProbe Inc., San Diego, CA).

*Mycobacterium avium* DNA was extracted by a previously described method.[5] Approximately 1 μg of the extracted DNA was digested with 15 units of PvuII (New England BioLabs, Beverly, MA) for 4 hours at 37°C, and analyzed by a Southern blot method on a piece of nylon membrane. The 475-bp PCR-amplified DNA was labelled with digoxigenin and used as a probe to hybridize to the IS*1245* sequences in the membrane. The bands were visualized by a color substrate detection system supplied by Boehringer Mannheim (Mannheim, Germany). Digoxigenin-labelled markers II and VII (Boehringer Mannheim) were used as molecular size markers in all of the membranes.

The DNA fingerprint patterns on the nylon membrane were detected visually and by computer software GelCompar (Applied Maths, Belgium). Following the convention established for the comparison of DNA fingerprint patterns generated from *M. tuberculosis* isolates, cluster pattern strains were defined as those strains having the same fingerprint pattern found in isolates from two or more patients.[8] Strains with more than five copies of IS*1245* whose patterns differed by one or two bands were defined as being closely related. Unique pattern isolates were those whose fingerprint pattern was not found among any of the other isolates.

### Data Analysis

Data entry and analysis were performed with the computer software EpiInfo version 5.01b (Centers for Disease Control and Prevention, Atlanta, GA, and World Health Organization, Geneva). Chi-square and Fisher's exact tests were used to compare proportional variables.

## RESULTS

### Demographic Features

A total of 137 *M. avium* isolates from 105 patients were analyzed. Seventy-two (69%) of the patients attended an AIDS outpatient clinic (Centro de Referencia e Treinamento em DST/AIDS or CRTA) or an infectious disease hospital (Instituto de Infectologia Emílio Ribas) in São Paulo; 22 (21%) attended hospitals in Rio de Janeiro, and 6 (5.7%) were from other states in Brazil (5 from Rio Grande do Sul and 1 from Ceará) (Table 1). Source data were not available for five patients. Most of the patients (80/105) had a diagnosis of AIDS (as defined above) at the time of specimen collection that led to the isolation of *M. avium*. Of all tested patients, two from Rio de Janeiro were HIV-seronegative. In both Rio de Janeiro and São Paulo, *M. avium* was isolated predominantly from male patients. Age information was available from only 56 (54%) of the patients (see Table 1).

### Strain Characteristics

All 137 strains biochemically defined as *M. avium* tested positive for a 427-bp fragment of the IS*1245* by PCR amplification. Interestingly, six of the strains failed to be detected by the *M. avium* or *M. intracellulare* AccuProbe Kits. Two of these had the same cluster DNA fingerprint pattern.

Among 137 isolates, 83 different banding patterns were observed. The copy number of IS*1245* ranged from three in three isolates to 26 in two isolates. Less than 4% of the isolates had IS*1245* copy numbers of five or less.

**Table 1.**  Patient Characteristics according to
Their *M. avium* IS*1245* DNA Fingerprint Patterns

| Characteristic | Cluster Pattern (n = 33 patients) | Noncluster Pattern (n = 72 patients) |
|---|---|---|
| State of origin | | |
| Rio de Janeiro | 12 | 10 |
| São Paulo | 16 | 56 |
| Other | 5 | 6 |
| Gender | | |
| Male | 23 | 47 |
| Female | 6 | 10 |
| Unknown | 4 | 15 |
| Age (y) | | |
| 2–15 | 2 | 1 |
| 16–35 | 5 | 25 |
| 36–50 | 10 | 10 |
| >50 | 0 | 3 |
| Unknown | 16 | 33 |
| HIV serostatus | | |
| Seropositive | 27 | 53 |
| Seronegative | 0 | 2 |
| Unknown | 6 | 17 |
| Site of isolation | | |
| Sterile* | 21 | 33 |
| Nonsterile† | 10 | 34 |
| Unknown | 2 | 5 |
| Year of isolation | | |
| 1990 | 2 | 15 |
| 1991–92 | 4 | 11 |
| 1993–94 | 21 | 37 |
| Unknown | 6 | 9 |

*Sterile sites included blood (n = 50 isolates), bone marrow (n = 10), lymph node (n = 3), and cerebrospinal fluid (n = 1). †Nonsterile sites included sputum (n = 45), bronchioalveolar lavage fluid (n = 6), stool (n = 8), urine (n = 4), skin (n = 2), gastric lavage (n = 1), and ear (n = 1). Six isolates had unknown site of isolation.

None of the isolates with identical IS*1245* patterns was noted to be isolated on the same day at Adolfo Lutz Mycobacteriology Laboratory, decreasing the possibility of a laboratory contamination.

### DNA Fingerprint Analysis and Sites of Isolation

The major sources of *M. avium* isolates were blood and sputum (see Table 1). Overall 65 (47%) of 137 isolates were recovered from sterile sites. Two isolates from a single patient were obtained at different time periods in 23 patients. In 18 such patients, one of the paired isolates was recovered from a nonsterile site, whereas the other was isolated from a sterile site. Among these isolates, identical fingerprint patterns were seen in 10 (56%) pairs, and distinct patterns were seen in 8 (44%). In two other patients, two isolates of *M. avium* were recovered only from blood at two different periods; one pair had the same pattern and the other showed distinct patterns. Three patients had multiple isolates with identical DNA fingerprint patterns at different periods from the same nonsterile sites.

Five (23%) of 22 patients from Rio de Janeiro had isolates with a cluster pattern designated JJJ, and two others showed a closely related pattern, designated JJJ1

(Figure 1). They were isolated from patients attending five different hospitals between May 1992 and May 1994. Among them, three JJJ pattern strains and one JJJ1 strain were isolated from blood, whereas the others were isolated from nonsterile sites. All patients infected with these strains were HIV seropositive and male. In addition, three other persons in Rio de Janeiro were infected with cluster pattern strains (H1, H3, and R). Hence, in Rio de Janeiro, 12 of 22 (55%) patients had isolates that belonged to one of these five clusters. Six of them had isolates recovered from sterile sites, and six from nonsterile sites (Table 2). No significant association was found between clusters and specimen source.

In contrast, only 16 of 72 (22%) patients from São Paulo had isolates that belonged to cluster pattern strains. However, these cluster pattern strains were significantly associated with isolation from sterile sites; 13 (81%) of 16 patients with cluster pattern strains compared to 27 (49%) of 55 patients with noncluster strains had these isolates recovered from sterile sites (P < 0.05; chi-square test with Yates correction). The site of isolation of one strain was unknown.

Five cluster strains from both cities (A, NNN, R, T, and ZZ) were isolated only from sterile sites (see Table 2). Strain A was isolated from blood samples from two different patients: one in December of 1992 and the other in February of 1993. Strain ZZ was isolated from blood in one patient in October 1991 and from bone marrow of another in May 1994. All four were seen at CRTA in São Paulo. Strain R was isolated in July 1993 from blood from one patient and from bone marrow in November 1994 from another patient. Both patients attended the same hospital in Rio de Janeiro. Information was not available for the other cluster pattern strains.

Among 11 *M. avium* patients from other states or of unknown source, two had isolates that shared DNA fingerprint patterns identical to those of isolates from Rio de Janeiro. Three of five patients from Rio Grande do Sul had isolates with a cluster pattern PP1. Others had unique pattern isolates. Two HIV-seronegative women had isolates with distinct patterns (JJ and UUU).

### DISCUSSION

*Mycobacterium avium* is known to be present in soil, water, and animals, but the association of environmental strains with disseminated infection in patients with AIDS has not been definitively demonstrated. Although certain serovars found in the environment can be isolated from cases of disseminated *M. avium* infections,[4] the serotyping method is not sensitive enough to conclude that patients with AIDS acquire infection from these environmental sources. To assess modes of transmission of *M. avium* infections, a more refined strain typing method is needed.
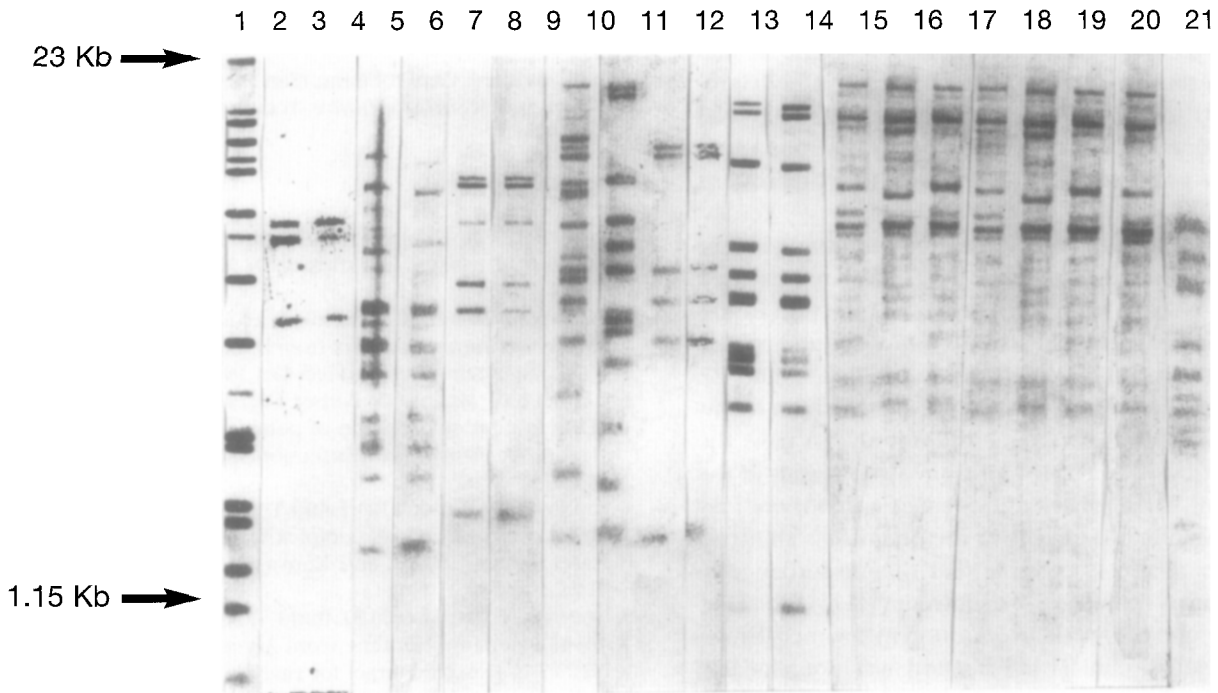
**Figure 1.** IS*1245* DNA fingerprint patterns of selected *M. avium* strains isolated from Brazilian patients. Lane 1, molecular size marker; lanes 2 and 3, pattern A; 4 and 5, ZZ; 6 and 7, B; 9, T; 10 and 11, C; 12 and 13, H1; 15 and 18, JJJ1; 14, 16, 17, 19, 20, JJJ; 21, R. Lane 8 is a noncluster pattern strain.

Other strain typing methods are used to discriminate *M. avium* strains, including PFGE and detection of insertion elements.[5,9-12] The IS*1245* has been reported to be found only among *M. avium* subspecies *avium*, *sylvaticum*, and *paratuberculosis*.[5] Hence, in patients with AIDS, the use of this insertion element not only is useful for typing strains but is helpful for distinguishing *M. avium* from *M. intracellulare* infections. In Brazil disseminated *M. avium* infection is an emerging AIDS-related

opportunistic disease. The authors had an opportunity to examine isolates from selected populations in two large cities in Brazil during this emerging period and believe that these isolates are representative of strains that infect AIDS patients, particularly in São Paulo. In São Paulo, *M. avium* isolates were obtained from two major institutions dedicated to the care of patients with AIDS. One is an outpatient center (CRTA) that sees 1000 to 1200 patients each year; the other is an infectious disease referral hospital with a 55-bed AIDS inpatient service (Instituto de Infectologia Emílio Ribas). A previous 2-year study (1990–1992), performed in the latter hospital, found *M. avium* in the bone marrow of 23 (18%) of 125 patients with AIDS with fever, anemia, and leukopenia.[2]

Based on low prevalence of positive skin test reactions to *M. avium* in the general population, and evidence of transmission from hospital water supply in one study, von Reyn et al have suggested that most *M. avium* infections that occur in patients with AIDS in industrialized countries like the United States result from recent infection as opposed to reactivation of an old infection.[3,13] In places like Brazil, where disseminated *M. avium* infections were not recognized until well into the AIDS epidemic,[2] this question of reactivation versus recent infection remained unanswered. If the interpretation of the IS*6110* restriction fragment length polymorphism (RFLP) patterns used for *M. tuberculosis* to distinguish

**Table 2.** Number of Patients Infected with Cluster Pattern *M. avium* Strains with the Indicated IS*1245* Fingerprint Patterns according to the Source of Isolates

| IS1245 Pattern | State | Total Number of Patients | Number with Isolate from a Sterile Site |
|---|---|---|---|
| A | SP | 2 | 2 |
| B | SP | 2 | 2 |
| B2 | SP | 2 | 1 |
| H1 | SP, RJ | 3 | 2 |
| H3 | RJ, RG | 4 | 0 |
| JJJ | RJ | 5 | 3 |
| JJJ1 | RJ | 2 | 1 |
| NNN | SP | 2 | 2 |
| PP1 | RG | 3 | 1 |
| R | RJ | 2 | 2 |
| S1 | SP | 2 | 1 |
| T | SP | 2 | 2 |
| ZZ | SP | 2 | 2 |

SP = São Paulo, RJ = Rio de Janeiro, RG = Rio Grande do Sul.

tuberculosis due to recent infection from reactivation can be applied to *M. avium*, the observation made in this study suggests that in large cities of Brazil, a high proportion of *M. avium* infections, especially in disseminated disease (defined as isolation of the organism from a sterile site) in patients with AIDS results from recent infection.

In São Paulo, although cluster pattern strains were isolated from only 22% of 72 patients, they represented 81% of the isolates from sterile sites, or infection in disseminated disease. In São Paulo or Rio de Janeiro, the cluster pattern strains designated A, NNN, R, T, and ZZ were exclusively isolated from sterile sites. A strain recovered from a respiratory source did not always have the same pattern as that recovered from a sterile site in the same patient. These observations suggest that there may be strain-to-strain differences in the organism's ability to disseminate. It also is possible that this discordance in isolate patterns represents bias introduced by the selection of only one colony of *M. avium* per specimen. Unfortunately, detailed clinical information was not available from these patients to correlate clinical symptoms with sources of isolates. The small number of isolates that were examined makes this conclusion preliminary.

The DNA fingerprint patterns appeared to be stable over time. The isolation of an identical pattern strain (R) several months apart from two patients admitted to the same hospital in Rio de Janeiro (who were not there at the same time) suggests that there may be a common source for this strain. This observation needs to be confirmed.

The recovery of different strains from blood at different time periods also indicates that a patient with AIDS may be reinfected even after treatment, or that such persons may have a polyclonal infection. In Brazil, *M. avium* prophylaxis with antibiotics is not widely practiced, and none of the patients in this study received prophylaxis.

The increasing incidence of *M. avium* infections in Brazil requires better understanding of the pattern and sources of transmission. The IS*1245*-based method to type strains appears to be as useful as the now standardized IS*6110*-based typing method used to study transmission of tuberculosis.[2] There is an opportunity to characterize this emerging problem in Brazil before the problem becomes widespread, as it already has in developed countries in North America and Europe.

## REFERENCES

1. Inderlied CB, Kemper CA, Bermudez LM. The *Mycobacterium avium* complex. Clin Microbiol Rev 1993; 6:266–310.
2. Barreto JA, Palaci M, Ferrazoli L, et al. Isolation of *Mycobacterium avium* complex from bone marrow aspirates of AIDS patients in Brazil. J Infect Dis 1992; 168:777–779.
3. von Reyn CF, Maslow JN, Barber TW, Falkinham JO III, Arbeit RD. Persistent colonization of potable water as a source of *Mycobacterium avium* infection in AIDS. Lancet 1994; 343:1137–1141.
4. von Reyn CF, Waddell RD, Eaton T, et al. Genetic diversity of *Mycobacterium avium* complex from water in the United States, Finland, Zaire, and Kenya. J Clin Microbiol 1993; 31:3227–3230.
5. Guerrero C, Barnasconi D, Burki T, Bodmer T, Telenti A. A novel insertion element from *Mycobacterium avium*, IS*1245*, is a specific target for analysis of strain relatedness. J Clin Microbiol 1995; 33:304–307.
6. David H, Levy-Frebault V, Papa F. Methodes de laboratoire pour mycobacteriologie clinique. Paris: Institut Pasteur, 1986.
7. Vestal AL. Procedures for the isolation and identification of mycobacteria. Washington, U. S. Department of Health Education and welfare. (HEW publication CDC 76-8230), 1976.
8. van Embden JAD, Cave MD, Crawford JT, et al. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for standardized methodology. J Clin Microbiol 1993; 31:406–409.
9. Arbeit RD, Slutsky A, Barber TW, et al. Genetic diversity among strains of *M. avium* causing monoclonal and polyclonal bacteremia in patients with the acquired immunodeficiency syndrome (AIDS). J Infect Dis 1993; 167: 1384–1390.
10. Hernandez Peres M, Fomukong NG, Hellyer T, Brown IN, Dale J. Characterization of IS*1110*, a highly mobile genetic element from *Mycobacterium avium*. Mol Microbiol 1994; 12:717–724.
11. Kunze ZM, Portaels F, McFadden JJ. Biologically distinct subtypes of *Mycobacterium avium* differ in possession of insertion sequence IS*901*. J Clin Microbiol 1994; 30:2366–2372.
12. Roiz MP, Palenque E, Guerrero C, Garcia MJ. Use of restriction fragment length polymorphism as a genetic marker for typing *Mycobacterium avium* strains. J Clin Microbiol 1995; 33:1389–1391.
13. von Reyn CF, Barber TW, Arbeit RD, et al. Evidence of previous infection with *M. avium* among healthy subjects: an international study of dominant mycobacterial skin test reactions. J Infect Dis 1993; 168:1553–1558.