# Mycobacterium haemophilum: Emerging or Underdiagnosed in Brazil?

**To the Editor:** *Mycobacterium haemophilum* was first described in 1978 by Sompolinsky et al. (1) as the cause of cutaneous infections in a patient with Hodgkin disease. Since then, fewer than 100 cases have been reported worldwide, mostly among immunocompromised patients (2), although *M. haemophilum* infection has also been described in immunocompetent patients as the cause of cervical, submandibular, and perihilar lymphadenopathy in children and of pulmonary nodules in an adult (3–5). Cases have been reported from United States, Australia, Canada, France, Israel, and the United Kingdom, but to date no reports have originated in South America.

The most frequent clinical sign of *M. haemophilum* infection in adults is a skin or joint lesion. Less common sites for isolation of *M. haemophilum* include the respiratory tract, blood, bone marrow, bone, and central venous catheters (2,6). *M. haemophilum* is unique among *Mycobacterium* species owing to its special growth requirements: it grows best at 30°C and requires an iron supplement (hemin or ferric ammonium citrate).

We report here the characterization of three strains of *M. haemophilum* isolated from patients living in three states in two distinct regions of Brazil, Rio de Janeiro and São Paulo (southeast region) and Bahia (northeast region). The first strain was detected in Rio de Janeiro in December 2000 from a blood culture of a 67-year-old man who had received a kidney transplant in 1988 at the age of 55 years and was undergoing immunosuppressive treatment with prednisolone and mycophenolate mofetil. The second strain was detected in São Paulo in March 2001 in a 43-year-old HIV-seropositive man from a biopsied specimen of a nasal ulcer. A direct acid-fast stain showed many acid-fast bacilli. At time of diagnosis, the patient's CD4+ cell count was 8/mm$^3$ and his viral load was 290.000 copies/mL. The third isolate was detected in Bahia in a 30-year-old HIV-seropositive man who had osteomyelitis in an elbow. A direct acid-fast stain showed rare acid-fast bacilli.

The isolate from the Rio de Janeiro patient grew only in Myco/F Lytic media (Becton Dickinson Microbiology Systems, Sparks, MD) plus blood in primary isolation and subculture; it failed to grow on chocolate agar at 30°C after 6 weeks. The isolates from São Paulo and Bahia showed a slight growth in 12B media on primary isolation; this growth was likely supported by the iron provided by the biopsied tissue. Subcultures on chocolate agar showed good growth after 2–3 weeks at 30°C. The isolates did not grow on Middlebrook 7H10 agar without hemin and grew on the same media when supplemented with 60 MM of hemin. Both strains showed a negative catalase reaction.

The species of all isolates was identified through polymerase chain reaction amplification of the gene encoding for the 65-kDa heat shock protein, followed by restriction analysis with the enzymes *Bst*EII and *Hae*III as described by Telenti et al. (7), with minor modifications. The three isolates showed the same restriction pattern as that obtained for *M. haemophilum* American Type Culture Collection 29548 prototype strain. Isolates from Rio de Janeiro and São Paulo were also molecularly characterized as previously described by Roth et al. (8), corroborating *M. haemophilum* species identification.

To our knowledge, these *M. haemophilum* isolates are the first to be reported in Brazil. These three patients came from cities 429–962 km apart, demonstrating the dispersion of *M. haemophilum* infection in Brazil. Given the specific requirements of *M. haemophilum* for its growth in culture, our findings suggest that its true incidence in Brazil is greatly underestimated. Consequently, we strongly recommend that clinical laboratories in Brazil include an iron-supplemented medium, such as chocolate agar, incubated at 30ºC, for primary isolation of *Mycobacterium* spp in samples from selected patients.

**Jorge Luiz Mello Sampaio,\*†
Venâncio Avancini Ferreira Alves,‡
Sylvia Cardoso Leão,†
Vanda Dolabela de Magalhães,§
Marinês Dalla Valle Martino,§
Caio Marcio Figueiredo
Mendes,\*Antonio Carlos de Oliveira
Misiara,¶ Kozue Miyashiro,\*
Jacyr Pasternak,§ Eliana
Rodrigues,‡ Ronaldo Rozenbaum,#
Carlos Alberto Sant´Anna Filho,\*\*
Sônia Regina Marques Teixeira,\*
Adriano Cunha Xavier,††
Mauro Silvério Figueiredo,\*
and José Paulo Gagliardi Leite††,‡‡**

\*Fleury - Centers for Diagnostic Medicine, São Paulo, Brazil; †Federal University of São Paulo, São Paulo, Brazil; ‡Oswaldo Cruz Hospital, São Paulo, Brazil; §Albert Einstein Hospital, São Paulo, Brazil; ¶Sírio Libanês Hospital, São Paulo, Brazil; #Servidores do Estado Hospital, Rio de Janeiro, Brazil;\*\*Aliança Hospital, Salvador, Bahia, Brazil; ††Lâmina Laboratory, Rio de Janeiro, Brazil; ‡‡Oswaldo Cruz Institute, Rio de Janeiro, Brazil

## References

1. Sompolinsky D, Lagziel A, Naveh D, Yankilevitz T. *Mycobacterium haemophilum* sp. nov., a new pathogen of humans. Int J Syst Bacteriol 1978;28:67–75.
2. Shah MK, Sebti A, Kiehn E, Massarella SA, Sepkowitz KA. *Mycobacterium haemophilum* in immunocompromised patients. Clin Infect Dis 2001;33:330–7.
3. Samra Z, Kaufmann L, Zeharia A, Ashkenazi S, Amir J, Bahar J, et al. Optimal detection and identification of *Mycobacterium haemophilum* in specimens from pediatric patients with cervical lymphadenopathy. J Clin Microbiol 1999;37:832–4.
4. Armstrong KL, James RW, Dawson DJ, Francis PW, Masters B. *Mycobacterium haemophilum* causing perihilar or cervical lymphadenitis in healthy children. J Pediatr 1992;121:202–5.
5. White DA, Kiehn TE, Bondoc AY, Massarella SA. Pulmonary nodule due to *Mycobacterium haemophilum* in an immuno-

competent host. Am J Respir Crit Care Med 1999;160:1366–8.

6. Ward MS Lam KV, Cannell PK, Herrmann RP. Mycobacterial central venous catheter tunnel infection: a difficult problem. Bone Marrow Transplant 1999;24:325–9.

7. Telenti A, Marchesi F, Balz M, Bally F, Bottger EC, Bodmer T. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J Clin Microbiol 1993;31:175–8.

8. Roth A, Reischl U, Streubel A, Naumann L, Kroppenstedt RM, Habicht M, et al. Novel diagnostic algorithm for identification of mycobacteria using genus-specific amplification of the 16S-23S rRNA gene spacer and restriction endonucleases. J Clin Microbiol 2000;38:1094–104.

Address for correspondence: Jorge Luiz Mello Sampaio, Fleury–Centers for Diagnostic Medicine, Microbiology Section, Av. General Waldomiro de Lima 508, São Paulo, Brazil 04344-070; fax: 55 11 50147601; e-mail: jorge.sampaio@fleury.com.br.

# Children and Multidrug-Resistant Tuberculosis in Mumbai (Bombay), India

**To the Editor:** India has the highest number of tuberculosis (TB) cases in the world. Each year in India, over 2 million new cases of TB are diagnosed, and approximately 500,000 persons die of the disease (1). During the last decade, multidrug-resistant TB has burgeoned in India, resulting in an extremely large number of multidrug-resistant TB cases, second only to the number of cases noted in Latvia (2). Since 1993, in response to this epidemic, the government of India has implemented the Revised National Tuberculosis Control Program, which is based on directly observed treatment (short course) principles (1).

Mumbai (formerly Bombay), India, is a densely populated metropolis with a population of approximately 12 million, 4.8 million (40%) of whom reside in overcrowded slums. Since 1990, a resurgence of TB has occurred, characterized by a 70% to 140% increase in the rate of TB-related deaths among adults aged 25–44 years (3). A vital factor contributing to this phenomenon is HIV infection. A recent review of autopsy reports from Mumbai showed that 85 (59%) of 143 adult patients with AIDS were diagnosed with pulmonary TB (4), indicating that the disease is the most common opportunistic infection for persons with AIDS. Commensurate with the increase in TB cases is a surge in the prevalence of multidrug-resistant TB in adult patients. Two reference mycobacterial laboratories in private hospitals in Mumbai have reported a high prevalence of multidrug-resistant TB strains; 56 (11%) of 521 cases in 1991–1995, and 58 (58%) of 100 cases in 1994–1995 (5,6).

The crisis of multidrug-resistant TB in adults in Mumbai has been well documented (7). However, little attention has been directed at children also affected by the resurgent TB epidemic. We think that TB is developing in more children in Mumbai today than a decade earlier. Moreover, close proximity to adult patients with multidrug-resistant TB makes children prone to developing primary multidrug-resistant TB, a vulnerability documented in a South African study (8). Similarly, disseminated TB is occurring in large numbers of children living in overcrowded slums in Mumbai with a consequent high death rate (9); we attribute many of these deaths in children to primary multidrug-resistant TB. However, this conclusion is difficult to document, as most affected children are sputum-negative for acid-fast bacilli. Contact tracing to detect the adult source of infection is routinely undertaken; often we can trace the source of infection. Because the facilities for culture and susceptibility testing are not available at affordable rates, proving that the adult contact has multidrug-resistant TB is not feasible in most cases.

The AIDS epidemic in adults in Mumbai has adversely affected the epidemic within the population of children with TB. HIV infection in young adults has resulted in a large number of HIV-infected infants, the result of a lack of any large-scale program aimed at preventing vertical transmission. To combat the growing problem with HIV-infected infants, India's National AIDS Control Organization is performing feasibility studies for implementing interventions to prevent mother-to-child transmission of HIV infection. Clinical trials with nevirapine are currently being conducted at five major public hospitals in Mumbai.

Multidrug-resistant TB frequently develops in adult AIDS patients (7). Accordingly, many pediatric AIDS patients in Mumbai are also developing primary multidrug-resistant TB. Since most families cannot afford antiretroviral therapy, HIV-infected children in whom TB is diagnosed are prescribed a four-drug TB treatment (consisting of isoniazid, rifampicin, pyrazinamide, and ethambutol) and co-trimoxazole for *Pneumocystis carinii* pneumonia prophylaxis. Although deaths in these children are being attributed to AIDS, we think that many of these deaths are related to multidrug-resistant TB.

To combat the TB epidemic, the Revised National Tuberculosis Control Program directly observed treatment strategy has been implemented as part of a public health program. However, most patients receive treatment from private physicians and thus remain outside the purview of the strategy. Private physicians seldom refer their patients to centers offering directly observed treatments because of potential for loss of income (3). In 1991, Uplekar and Shepard (10) reported that 100 private physicians in the Dharavi slums in Mumbai prescribed 80 different anti-TB regimens; most were both inappropriate and expensive. Since private physicians have not yet been involved in the gov-