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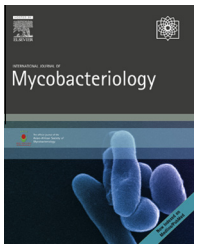

Short Communication

First detection of *Mycobacterium triplex* in Latin America

Carlos Eduardo Dias Campos [a], *Cláudia Fontoura Dias* [b], *Gisela Unis* [b], *Paulo Cesar de Souza Caldas* [a], *Paulo Redner* [a], *Luciana Distásio de Carvalho* [a], *Ana Paula Chaves Sobral Gomes* [a], *Marta Osório Ribeiro* [c], *Fátima Cristina Onofre Fandinho Montes* [a], *Enrico Tortoli* [d], *Jesus Pais Ramos* [a,*]

[a] *National Reference Laboratory for Tuberculosis, Centro de Referência Professor Hélio Fraga, Escola Nacional de Saúde Pública, Fiocruz, Rio de Janeiro, Brazil*

[b] *Hospital Sanatório Partenon, Secretaria Estadual da Saude, Porto Alegre, Rio Grande do Sul, Brazil*

[c] *Fundação Estadual de Produção e Pesquisa em Saúde, Porto Alegre, Rio Grande do Sul, Brazil*

[d] *Emerging Bacterial Pathogens Unit, San Raffaele Scientific Institute, Milan, Italy*

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ABSTRACT

In this study we describe the first isolation of *Mycobacterium triplex* in Latin America. This species causes infections in humans, with very few reports from around the world. We isolated two sputum specimens of a patient with a 6-year history of human immunodeficiency and tuberculosis treatment failure. All tests used confirmed *M. triplex* and the patient responded well to drug therapy for 18 months.

Introduction

Nontuberculous mycobacteria are ubiquitous and transmitted to humans through the environment from sources like water and soil, causing infections in different areas of the body, but mainly pulmonary, skin, soft tissue infections, and lymphadenitis [1].

Mycobacterium triplex is a slow growing, nonpigmented, nontuberculous mycobacterium, described in 1996 from clinical strains of different geographical locations in the United States [2]. This species is related to *Mycobacterium simiae*, *Mycobacterium lentiflavum*, and *Mycobacterium sherrisii*, but more closely to *Mycobacterium genavense* [3].

Only a few cases of *M. triplex* infections have been reported in the United States and Europe. To our knowledge, this is the first report of *M. triplex* infection in a country of Latin America. This study was approved by the ENSP–Fiocruz Committee for Ethical Research with human beings.

* Corresponding author at: National Reference Laboratory for Tuberculosis, Estrada da Curicica 2000, Rio de Janeiro, RJ 22780192, Brazil.
E-mail address: jepramos@ensp.fiocruz.br (J.P. Ramos).

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Methods

The patient studied was clinically, laboratorial, and radiological assessed at diagnosis. Monthly cultures were performed to monitor the progress of treatment in Hospital Sanatório Partenon in Porto Alegre, Rio Grande do Sul state, Brazil.

Two sputum isolates were obtained according of American Thoracic Society microbiologic criteria for nontuberculous mycobacterial lung disease [4] and processed at the central laboratory of Rio Grande do Sul, Brazil, in the period of 2007–2008. After growth in Löwenstein–Jensen medium, the cultures were sent to the Centro de Referência Professor Hélio Fraga/ENSP/FIOCRUZ for identification, but the PRA-*hsp65* methodology did not allow for identification at species level.

To clarify the identity and to characterize the isolates, phenotypic and sequencing assays were performed in the laboratory of Centro de Referência. The two isolates were designated as HF1226 and HF2396 and tested for pigment production and growth rate at 35–37 °C. The following biochemical tests were performed: nitrate reduction, niacin production, heat-stable catalase (68 °C), arylsulfatase activity (at 3 days), Tween 80 hydrolysis, and urease. The isolates were slow growing and nonpigmentated. The biochemical profile was positive for nitrate reduction, heat-stable catalase, and urease, and negative for niacin, 3 days arylsulfatase, and Tween 80 hydrolysis. Part of the RNA polymerase beta-subunit gene (*rpoB*), the 16S–23S internal transcribed spacer, and the 441 bp fragment of the 65 kDa heat shock protein gene (*hsp65*) were sequenced using previously published approaches [5–7].

Results and discussion

The 51-year-old Caucasian woman studied had been treated for tuberculosis three times in the past. The patient presented with a productive cough and night sweats and her CD4 count was 514 cells/mL. She was being treated for human immunodeficiency virus with antiretroviral therapy (zidovudine, lamivudine, and tenofovir). The sputum smear microscopy showed acid-fast bacilli (+) in two samples. A chest X-ray revealed bilateral pulmonary lesions (nodular opacities and cavities). She was empirically prescribed amikacin, ofloxacin, ethambutol, and clarithromycin, since there was no identification of mycobacteria. No sensitivity test was conducted. During follow-up, her respiratory symptoms improved and periodic respiratory specimen cultures tested negative. She was considered cured after 18 months of treatment.

The 16S rDNA gene was sequenced using MicroSEQ Full Gene 16S rDNA Kit (Life Technologies, Carlsbad, CA, USA), as indicated by the manufacturer. All the sequences were compared on identity with the ones of the closest reference strains using a basic local alignment search tool (BLAST) search [8]. The sequences of reference type strains (retrieved from GenBank) and strain HF1226 (the older of the two isolates) were used to build a concatenated neighbor-joining tree [9] using the MEGA version 6 program [10]. Comparison with the reference strains present in GenBank through a BLAST search showed that the 16S rDNA (accession number KF856620), *hsp65* (KF856621), and internal transcribed spacer (KF856623) sequences of the isolates have 100% identity with *M. triplex* type strain (ATCC 700071), but the *rpoB* (KF856622) gene presented two mismatches when compared with *M. triplex* type strain (99.72% identity). The next high similarity was that of *Mycobacterium florentinum* type strain DSM 44852 with nine mismatches and 95.92% identity. The phylogenetic tree revealed that strain HF1226 was clustered (with 100% bootstrap) to the type strain of *M. triplex* and distinct from other related species (Fig. 1).

All phenotypic results were the same as described for the *M. triplex* type strain [2]. The molecular and phylogenetic analysis of the two isolates from the Brazilian patient confirmed the identification as *M. triplex*. BLAST analysis also revealed that the polymorphisms identified in the *rpoB* gene of these two strains had never been described, as no identical sequence was found in the database.

M. triplex has been suggested as a cause of disease in severely immunocompromised patients, but can also affect immunocompetent individuals [11,12]. Symptoms and radiological findings of the studied patient are similar to those in previous reports. There are few reported cases of

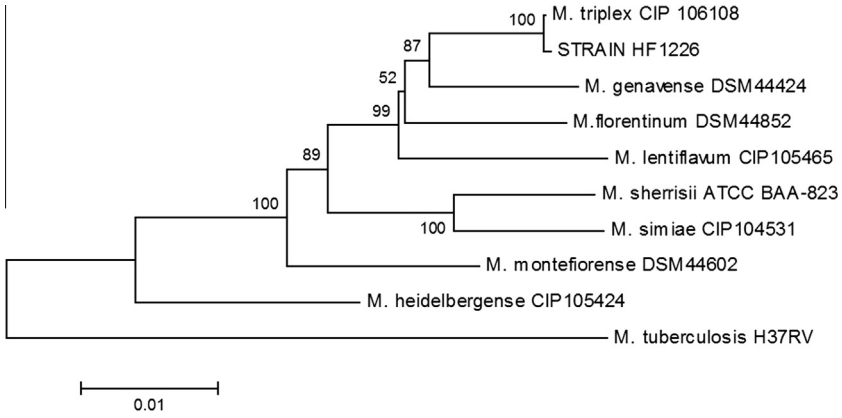

Figure 1 – Phylogenetic concatenated tree using 16S rDNA, *hsp65*, *rpoB*, and internal transcribed spacer sequences, constructed using neighbor-joining method and Kimura's two-parameter as the substitution model. The bootstrap values, calculated on 1000 replicates, indicated the significance of branches. *Mycobacterium tuberculosis* sequence was used as the outgroup. M. = *Mycobacterium*.

M. triplex infections to date. In Europe, *M. triplex* has been reported as causing pulmonary infection in patients of countries such as Italy, Portugal, Ireland, and Finland [12–15]. In the United States, a study described a case of *M. triplex* that was isolated from the pericardial and peritoneal fluid of a liver transplant patient [16]. Also, in the United States another study identified eight *M. triplex* strains from clinical *M. simiae* complex isolates [17]. There are extremely rare reports of patients infected by *M. triplex* outside Europe and the United States; one exception is a Nigerian human immunodeficiency virus positive man with central nervous system involvement [18].

The results presented in this study extend the range of geographical distribution of *M. triplex*. This finding increases the possibility of the diagnosis of new cases of *M. triplex* infection in Latin America, especially in Brazil.

Conflicts of interest

The authors have no conflict of interest.

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REFERENCES

[1] M.M. Johnson, J.A. Odell, Nontuberculous mycobacterial pulmonary infections, J. Thorac. Dis. 6 (2014) 210–220.
[2] M.M. Floyd, L.S. Guthertz, V.A. Silcox, et al, Characterization of an SAV organism and proposal of *Mycobacterium triplex* sp. nov., J. Clin. Microbiol. 34 (1996) 2963–2967.
[3] J. van Ingen, E. Tortoli, R. Selvarangan, et al, *Mycobacterium sherrisii* sp. nov., a slow-growing non-chromogenic species, Int. J. Syst. Evol. Microbiol. 61 (2011) 1293–1298.
[4] D.E. Griffith, T. Aksamit, B.A. Brown-Elliott, et al, ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases, Am. J. Respir. Crit. Care Med. 175 (2007) 367–416.
[5] T. Adékambi, P. Colson, M. Drancourt, *RpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria, J. Clin. Microbiol. 41 (2003) 5699–5708.
[6] A. Roth, M. Fischer, M.E. Hamid, et al, Differentiation of phylogenetically related slowly growing mycobacteria based on 16S–23S rRNA gene internal transcribed spacer sequences, J. Clin. Microbiol. 36 (1998) 139–147.
[7] A. Telenti, F. Marchesi, M. Balz, et al, Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis, J. Clin. Microbiol. 31 (1998) 175–178.
[8] S.F. Altschul, W. Gish, W. Miller, et al, Basic local alignment search tool, J. Mol. Biol. 215 (1990) 403–410.
[9] G. Devulder, M. Pérouse de Montclos, J.P. Flandrois, A multigene approach to phylogenetic analysis using the genus *Mycobacterium* as a model, Int. J. Syst. Evol. Microbiol. 55 (2005) 293–302.
[10] K. Tamura, G. Stecher, D. Peterson, et al, MEGA6: molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol. 30 (2013) 2725–2729.
[11] G. Caruso, R. Angotti, F. Molinaro, et al, Cervical lymphadenitis by *Mycobacterium triplex* in an immunocompetent child: case report and review, Indian J. Microbiol. 53 (2013) 241–244.
[12] C. Piersimoni, P. Zitti, G. Mazzarelli, et al, *Mycobacterium triplex* pulmonary disease in immunocompetent host, Emerg. Infect. Dis. 10 (2004) 1859–1862.
[13] A. Amorim, R. Macedo, A. Lopes, et al, Non-tuberculous mycobacteria in HIV-negative patients with pulmonary disease in Lisbon, Portugal, Scand. J. Infect. Dis. 42 (2010) 626–628.
[14] R. McMullan, J. Xu, M. Kelly, et al, *Mycobacterium triplex* pulmonary infection in an immunocompetent patient, J. Infect. 44 (2002) 263–264.
[15] S. Suomalainen, P. Koukila-Kähkölä, E. Brander, et al, Pulmonary infection caused by an unusual, slowly growing nontuberculous *Mycobacterium*, J. Clin. Microbiol. 39 (2001) 2668–2671.
[16] E. Hoff, M. Sholtis, G. Procop, et al, *Mycobacterium triplex* infection in a liver transplant patient, J. Clin. Microbiol. 39 (2001) 2033–2034.
[17] J. van Ingen, S.E. Totten, L.B. Heifets, et al, Drug susceptibility testing and pharmacokinetics question current treatment regimens in *Mycobacterium simiae* complex disease, Int. J. Antimicrob. Agents 39 (2012) 173–176.
[18] V. Zeller, A.L. Nardi, C. Truffot-Pernot, et al, Disseminated infection with a mycobacterium related to *Mycobacterium triplex* with central nervous system involvement associated with AIDS, J. Clin. Microbiol. 41 (2003) 2785–2787.