

## Letter to the Editor

Frederic Ballester, Fernando Alcaide, Isabel Pujol, Salvador Hernández-Flix, Josep M. Simó, Jorge Joven and Jordi Camps\*

# First human isolate of *Mycobacterium madagascariense* in the sputum of a patient with tracheobronchitis

**Keywords:** infectious diseases; mycobacteria; tracheobronchitis.

\*Corresponding author: **Jordi Camps**, Centre de Recerca Biomèdica, Hospital Universitari de Sant Joan, C. Sant Joan s/n, 43201 Reus, Catalonia, Spain, Phone: +34 977 310300, E-mail: jcamp@grupsagessa.com

**Frederic Ballester:** Laboratori de Referència de Catalunya SUD, Hospital Universitari de Sant Joan, Reus, Catalonia, Spain

**Fernando Alcaide:** Department of Microbiology, IDIBELL-Hospital Universitari de Bellvitge, Department of Pathology and Experimental Therapeutics, University of Barcelona, Barcelona, Catalonia, Spain; and Red Española de Investigación en Patología Infecciosa (REIPI), Instituto de Salud Carlos III, Madrid, Spain

**Isabel Pujol:** Laboratori de Referència de Catalunya SUD, Hospital Universitari de Sant Joan, Reus, Catalonia, Spain

**Salvador Hernández-Flix:** Department of Internal Medicine, Hospital Universitari de Sant Joan, Reus, Catalonia, Spain

**Josep M. Simó:** Laboratori de Referència de Catalunya SUD, Hospital Universitari de Sant Joan, Reus, Catalonia, Spain

**Jorge Joven:** Unitat de Recerca Biomèdica, Hospital Universitari de Sant Joan, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Reus, Catalonia, Spain

**Jordi Camps:** Unitat de Recerca Biomèdica, Hospital Universitari de Sant Joan, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, Reus, Catalonia, Spain

Reports on the isolation and identification of unusual *Mycobacteria* species in humans, animals and plants have increased considerably recently due, largely, to the implementation of Molecular Biology methods which have higher discriminative powers than the classical phenotype-based techniques [1]. Since the year 2000, several authors have identified a variety of *Mycobacteria* in clinical specimens, including *M. elephantis* [2, 3], *M. branderi* [4], *M. monacense* [5, 6], and *M. poriferae* [7].

*M. madagascariense* was first described by Kazda et al. [8]. It grows rapidly at temperatures ranging between 22°C and 31°C and is scotochromogenic [1]. Phylogeny studies based on DNA sequence classify *M. madagascariense*

within the group of thermo-tolerant rapid growers, phylo-genetically close to *M. confluentis* [9]. Experimental studies in animals indicate that this microorganism is not a pathogen [1]. To date, *M. madagascariense* has not been identified in any human sample. The present letter reports, to the best of our knowledge for the first time in the literature, the identification of *M. madagascariense* in the sputum of a patient with tracheobronchitis.

In August 2009, a 22-year-old Russian male was admitted to the Pneumology Department of the Hospital Universitari Sant Joan de Reus (Catalonia, Spain). He complained of a cough with purulent sputum dating from a year previously, with bloody sputum on occasions. He had no evidence of fever or toxic syndrome. Standard biochemical and hematological tests were normal. Serology for hepatitis B and C and human immunodeficiency virus (HIV) infection were negative. Thorax X-ray showed calcified adenopathies in the right mediastinum and hilum, indicating previous tuberculosis. Three serial sputum samples were collected and Zhiel-Neelsen staining was negative. A standard culture in agar plates was negative for bacterial respiratory pathogens. Cultures in Lowenstein-Jensen (LJ) medium with pyruvate (but not LJ medium alone) showed, 7 days later, abundant mucous, 2 mm sized, acid-fast, orange colonies, that were composed of Gram-positive rods. Acid fast bacilli staining was performed in the sputum samples before and after decontamination, and prior to inoculation into the LJ medium. These colonies were identified as *M. madagascariense* using molecular and phenotypic methods in a reference laboratory at the Hospital Universitari de Bellvitge (Barcelona, Spain). Currently, this mycobacterial species cannot be identified by routine commercial tests since the organism is not recognized with the available diagnostic systems. Although growth and chromogenic characteristics as well as biochemical tests (Table 1) were important for microbiology diagnosis, definitive identification relied on partial 16S rRNA gene sequencing (first 500 bp) [10]. The patient was

Characteristics	Result
Colony morphology	Smooth
Pigmentation	Scotochromogenic
Growth	
<7 days	Positive
22°C	Positive
31°C	Positive
37°C	Negative
Enzymatic activities	
Arylsulfatase (3 days)	Positive
Urease	Positive
Nitrate reductase	Negative
Tween hydrolysis (10 days)	Positive
Pyrazinamidase	Positive
NaCl (5%) tolerance	Negative
Iron uptake	Positive
Carbohydrate utilization	
D-Glucose	Positive
L-Arabinose	Positive
D-Fructose	Positive
D-Mannitol	Positive

**Table 1** Phenotypic characteristics of *M. madagascariense*.

treated for his tracheobronchitis with amoxicillin/clavulanate with favorable evolution and was referred back to the primary care physician for monitoring.

The source of this *M. madagascariense* infection could not be ascertained from questioning the patient or from the data available in the medical history. This bacterium was first described after the isolation of three strains from several species of sphagnum moss found between Toalanaro and Saint-Luce in the coastal region of Madagascar [8]. Sphagnum is widely distributed in the Northern Hemisphere, especially in tundra regions,

since it is used as a soil conditioner and as a building insulating material. Hence, the possibility exists that the infection was acquired by the patient in his country of origin. *M. madagascariense* is considered non-pathogenic [1] and, as such, an association between the presence of this bacterium and the pathophysiology of the tracheobronchitis is questionable. It is possible that the patient was contaminated by a few bacterial cells acquired from his environment. It needs to be noted that all mycobacteria are very resistant to bactericidal activity of macrophages and that, especially in the presence of a concomitant infection, might be very difficult to clear. It is also possible that the patient was allergic to sphagnum and the allergic reaction was related to the tracheobronchitis. However, all these considerations do not rule out a possible role of *M. madagascariense* in human pathology, especially in immunocompromised patients.

**Acknowledgments:** Editorial assistance was provided by Dr. Peter R. Turner of Tscimed.com.

## Conflict of interest statement

**Authors' conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article.

**Research funding:** None declared.

**Employment or leadership:** None declared.

**Honorarium:** None declared.

Received May 23, 2012; accepted July 25, 2012; previously published online September 12, 2012

## References

- Tortoli E. Impact of genotypic studies on Mycobacterial taxonomy: the new Mycobacteria of the 1990s. *Clin Microbiol Revs* 2003;16:319–54.
- Turenne C, Chedore P, Wolfe J, Jamieson F, May K, Kabani A. Phenotypic and molecular characterization of clinical isolates of *Mycobacterium elephantis* from human specimens. *J Clin Microbiol* 2002;40:1230–6.
- Potters D, Seghers M, Muydermans G, Piérard D, Naessens A, Lauwers S. Recovery of *Mycobacterium elephantis* from sputum of a patient in Belgium. *J Clin Microbiol* 2003;41:1344.
- Sugawara I, Kazumi Y, Otomo K, Ooki K, Mitarai S, Mori K. *Mycobacterium branderi* isolated from pus of a right pulmonary cavitory lesion. *Jpn J Infect Dis* 2005;58:187–8.
- Reisch U, Melzl H, Kroppenstedt RM, Miethke T, Naumann L, Mariottini A, et al. *Mycobacterium monacense* sp. nov. *Int J Syst Evol Microbiol* 2006;56:2575–8.
- Hogardt M, Schreff AM, Naumann L, Reischl U, Sing A. *Mycobacterium monacense* in a patients with a pulmonary tumor. *Jpn J Infect Dis* 2008;61:77–8.
- Ballester F, Pujol I, Alcaide F, Pizarro I, Simó JM, Joven J, et al. First human isolate of *Mycobacterium poriferae* in the sputum of a patient with chronic bronchitis. *J Clin Microbiol* 2011;49:3107–8.
- Kazda J, Müller HJ, Stackebrandt E, Daffe M, Müller K, Pitulle C. *Mycobacterium madagascariense* sp. nov. *Int J Syst Bacteriol* 1992;42:524–8.
- Kirschner P, Teske A, Schröder KH, Kroppenstedt RM, Wolters J, Böttger EC. *Mycobacterium confluentis* sp. nov. *Int J Syst Bacteriol* 1992;42:257–62.
- Harmsen D, Dostal S, Roth A, Niemann S, Rothgänger J, Sammeth M, et al. RIDOM: comprehensive and public sequence database for identification of *Mycobacterium* species. *BMC Infect Dis* 2003;3:26.