CASO CLÍNICO / *CLINICAL CASE*

O primeiro caso de infeção por *Mycobacterium heckeshornense* em Portugal

The first Mycobacterium heckeshornense infection reported in Portugal

/ J. Zelinová[1] / M. Custódio[1] / S. Alves[1,2] / M. Guimarães[2] / A. Marques[1,2] / V. Castro[1,2] / I. Vaz Pinto[2] / C. Santos[2]

[1] Department of Internal Medicine Cascais Hospital

[2] HIV/AIDS Unit of Cascais Hospital

Patrocínios:

O presente estudo não foi patrocinado por qualquer entidade

Artigo recebido em
06/03/2017

Artigo aceite para publicação em
06/04/2017

/ Resumo

A infeção por *Mycobacterium heckeshornense* (*M. heckeshornense*) é uma causa rara de doença broncopulmonar no Homem. Trata-se de uma micobactéria não-tuberculosa (MNT) de crescimento lento, fenotipicamente semelhante ao *Mycobacterium xenopi* (*M. xenopi*). Devido ao seu período de incubação prolongado, os métodos de isolamento padrão podem não detetar a sua presença, fazendo com que nem todos os casos sejam identificados.

Os autores relatam o primeiro caso em Portugal de infeção pulmonar por *M. heckeshornense* num paciente infetado por VIH, com doença pulmonar obstrutiva crónica agravada.

O tratamento ideal para a infeção por *M. heckeshornense* não está ainda definido, no entanto a sua semelhança com o *M. xenopi* sugere que um tratamento idêntico seja efetivo.

O doente iniciou o tratamento atualmente recomendado para *M. xenopi* tendo-se verificado melhoria após 4 meses.

Devido à escassez de informações na literatura, apresenta-se neste artigo uma breve revisão.

Palavras-chave: micobactéria não tuberculosa, *M. heckeshornense*, *M. xenopi*, VIH

/ Abstract

Mycobacterium heckeshornense *infection is a rare cause of bronchopulmonary disease in humans. This is a slow-growing nontuberculous mycobacteria (NTM), phenotypically related to* Mycobacterium xenopi*. Due to its prolonged incubation period, detection may be missed by standard mycobacterial isolation instruments causing it to be misidentified.*

The authors report the first case in Portugal of M. heckeshornense *pulmonary infection in an HIV-infected patient, with aggravated chronic obstructive pulmonary disease.*
Optimal treatment for M. heckeshornense *infection has not been established, however its resemblance to* M. xenopi *suggests that similar treatment is reasonable. Our patient initiated the current recommended treatment and four months later we saw improvement.*
Due to insufficient information in current literature we present a brief revision in this article.

Keywords: *Non-tuberculousmycobacteria,* heckeshornense, xenopi*, cavitation, HIV*

/ Introduction

Mycobacterium heckeshornense is a non-tuberculous mycobacterium species, first described in 2000.[1-3]

This mycobacteria is a slow-growing scotochromogen, that resembles *M. xenopi* due to its phenotypically and phylogenetically related characteristics. [1-7]

Infection due to *M. heckeshornense* is rare in humans[4] and there are limited reports of its isolation as a pathogen in immunocompetent patients [1], however the burden of human disease caused by *M. heckeshornense* may have been underestimated in the past due to misidentification as *M. xenopi* [2-7]. Published data is limited, in the 2014 inventory study of non-tuberculous mycobacteria in the European Union we can see that only eight cases were reported.[8]

/ Case Report

We report the first case of *M. heckeshornense* pulmonary infection in Portugal, in an HIV infected patient with good long-term immunological control.

A 66 year old man, smoker with extensive emphysema, was diagnosed in 2011 after an episode of pneumothorax, without any previous history of pulmonary tuberculosis. He also had HIV-1 infection diagnosed in 2003, at the moment with undetectable viral load and a CD4 lymphocyte count of 792cells/µL (38.5%). He's being treated with darunavir/ritonavir, tenofovir and emtricitabine.

Because of progressive asthenia to minimal effort he did a chestX-Ray which showed an irregular hypotransparency on the upper side of the right lung field (image 1). Chest computed tomography (CT) revealed "confluence of consolidation areas with cavitation in the apex of the right upper lobe, as well as severe centrilobular emphysema bullosa dystrophy in lung apex" (image 2A).

Direct acid-fast bacilli (AFB) smear examination of sputum and bronchoalveolar lavage (BAL) was negative. Fluid BAL culture was positive after twenty-eight days, using the rapid mycobacterium detection method with liquid mycobacterium growth bottle medium - *VersaTrek Myco System*.

The DNA strip assay - *GenoType Mycobacterium CM* ("common mycobacteria"- *M. tuberculosis complex, M. xenopi, M. intracellulare, M. avium, M. gordonae*, among others) was applied on specimens from cultivated material but it was negative, so after that it was done *GenoType Mycobacterium AS* ("additional species"- *M. simiae, M. haemophilum, M. kansasii, M. heckeshornence* among others) that did the diagnosis of *M. heckeshornense* infection.

The drug susceptibility test was performed by using a broth microdilution (MIC) method (*Sensititre Slow Growing Mycobacterium MIC Plate* - SLOMYCO) that tests drug susceptibility for thirteen antimicrobial agents and in this case demonstrated only doxycycline resistance.

The patient began treatment regimen with isoniazid, rifabutin, ethambutol and clarithromycin.

After four months of therapy, there was clinical and radiological improvement with the CT scan showing less consolidation with bigger residual lung cavities.

/ Discussion

Mycobacteria are immobile, slow-growing rod-shaped, gram-positive bacteria with high genomic G+C content (61-71%). Due to their special staining characteristics under the microscope, which is mediated by mycolic acid in the cell wall, they are called acid-fast.

Mycobacteria can be divided into three groups, *Mycobacterium tuberculosis complex* (causative pathogen of tuberculosis - TB), Nontuberculous mycobacteria (NTM) and *Mycobacterium leprae* (causative pathogen of leprosy).

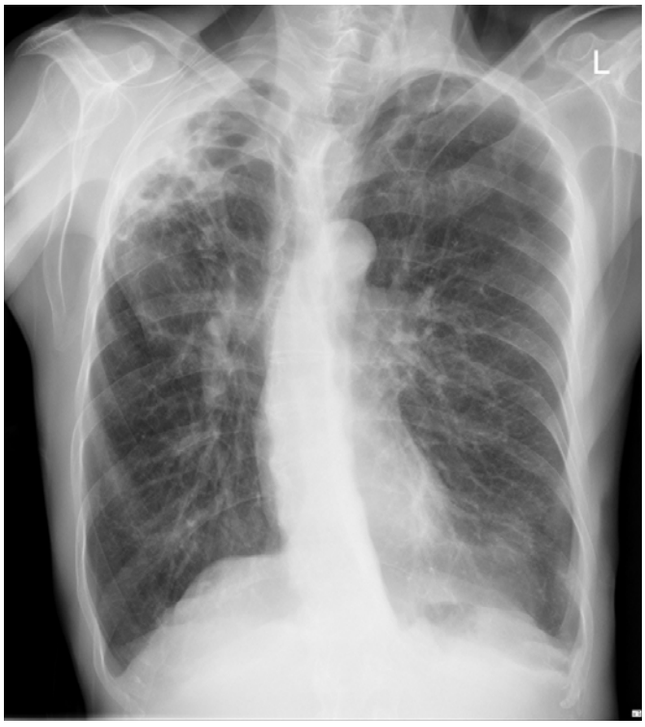

Image 1 – Chest X-Ray - Irregular hypotransparency on the upper side of the right lung field

The group of NTM, formerly called atypical or ubiquitous mycobacteria, contains over 150 species that shows a broad diversity regarding where they can be found and how they adapt to certain environmental conditions.

In contrast to *M. tuberculosis*, NTM are opportunistic pathogens that can cause distinctive clinical patterns in immunocompromised patients or patients with pre-existing pulmonary disease.

Human disease is suspected to be acquired from environmental exposures, although the specific source of infection usually cannot be identified. So far there is very limited evidence for person-to person transmission of NTM.[8,9]

M. heckeshornense was firstly reported in 2000 and since then, a limited number of cases were reported in humans.[1-3] *M. heckeshornense* is phenotypically very similar to *M. xenopi*, therefore, the burden of human disease caused by *M. heckeshornense* may have been underestimated in the past due to misidentification as *M. xenopi*.[2]

The treatment of nontuberculous mycobacteria infection is often complex and depends on the particular mycobacteria species so, the distinction between TB pathogens and NTM is known to be essential for diagnosis and treatment.

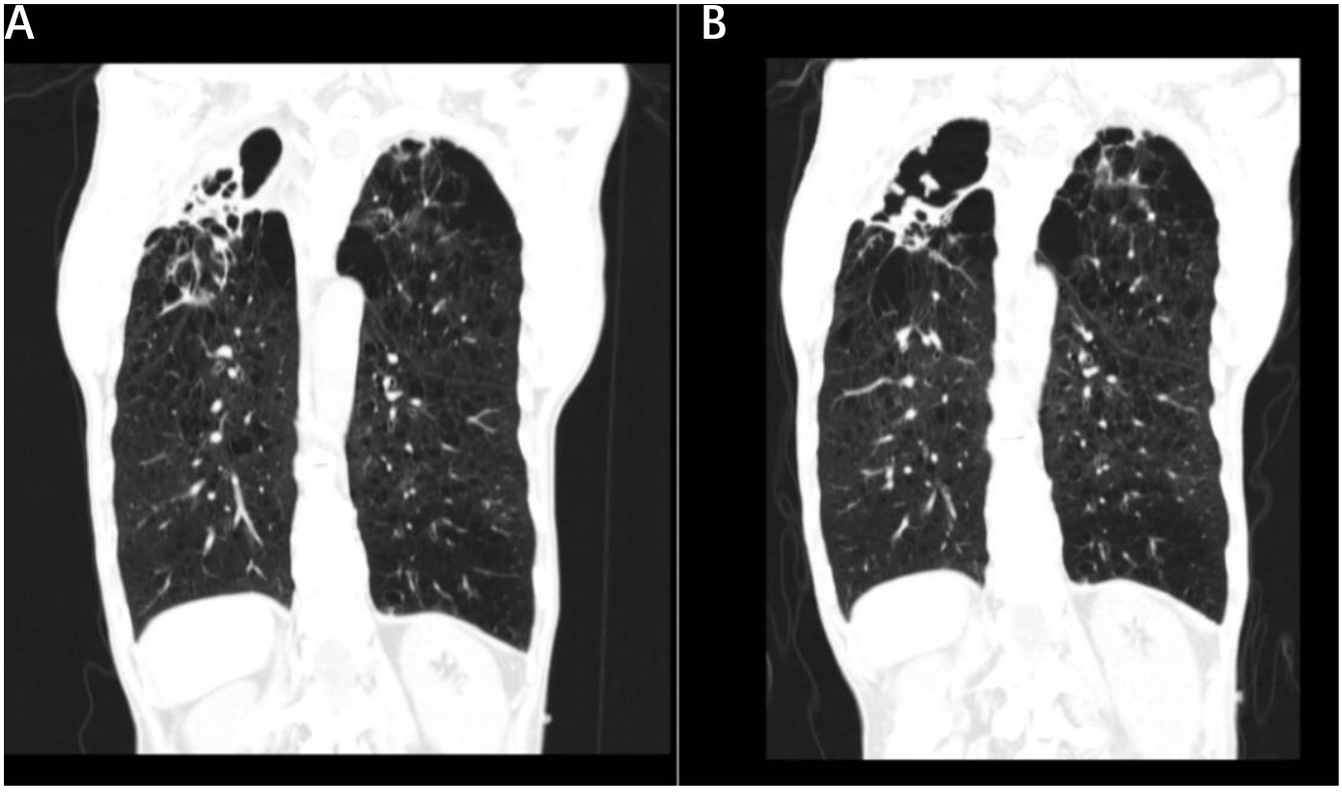

Image 2 – CT scan: A - confluence of consolidation areas with cavitation in the apex of the right upper lobe, as well as severe centrilobular emphysema bullosa dystrophy in lung apex. B - bigger residual lung cavities but less consolidation

In our case the medium used for the BAL culture was VersaTREK System that combines a liquid culture medium (VersaTREK Myco media), a growth supplement (Myco GS), and, for potentially contaminated species, an antibiotic supplement (Myco AS or Myco PVNA) with detection system that automatically incubates and continuously monitors culture bottles inoculated with specimens possibly containing mycobacteria.[10] Despite this, the culture was positive only after 28 days.

New molecular genetics methods for species differentiation are valuable tools in NTM diagnostics and offer advantages compared to time-consuming conventional methods.

This PCR-based test system is reliable for differentiation between the *M. tuberculosis* simplex and NTM, from cultivated material (solid or liquid). These tests are based on a PCR technique targeting a 23SrRNA gene region, followed by reverse hybridization and line probe technology (Image 3).

GenoType Mycobacterium CM ("common mycobacteria") can detect 27 different clinically relevant NTM and *GenoType Mycobacterium AS* ("additional species") detects 19 additional clinically relevant NTM. Both test systems can easily be combined (done separately or in combination) and therefore saving time and effort (results are obtained within only five hours).[11]

All mycobacteria should be tested for drug susceptibility. In our case this was done with the use of *Sensititre Slow Growing Mycobacterium MIC Plate* (SLOMYCO) that is a broth microdilution (MIC) method, that tests drug susceptibility for amikacin, ciprofloxacin, clarithromycin, doxycycline, ethambutol, ethionamide, isoniazid, linezolid, moxifloxacin, rifabutin, rifampin, streptomycin, trimethoprim/sulfamethoxazole. It can provide results up to 14 days for slow growing organisms.[12]

The limited reports regarding *M. heckeshornense* in the current medical literature make it impossible to provide definitive information on the treatment of infections with this organism.

Given the phenotypic similarities to *M. xenopi*, similar treatment may be reasonable, current recommendations include isoniazid, rifampin or rifabutin, and ethambutol, with or without an initial phase of streptomycin.[2,4,6] For HIV-infected patients, a prolonged treatment should be considered, similar to that of disseminated *Mycobacterium Avium Complex* infection, generally more than twelve months and accompanied by a sustained (more than six months) increase in CD4 counts above 100cells/µL.[4].

/ Conclusion

The authors reported the first Portuguese case of cavitary *M. heckeshornense* pulmonary disease. A treatment with isoniazid, rifabutin, ethambutol and clarithromycin led to clinical improvement.

The treatment of NTM is often complex and depends on the particular mycobacteria species, the distinction between tuberculosis (TB) pathogens and NTM is known to be essential for diagnosis and treatment.

Because of its prolonged incubation time, the incidence of *M. heckeshornense* infection may be underestimated.

New technologies, optimized culture medium (liquid or solid), with new automated technology systems and new molecular genetic methods for species differentiation are valuable tools in NTM diagnostics and offer advantages compared to time-consuming conventional methods.

Due to their variable susceptibility to first-line antituberculosis drugs, all mycobacteria should be tested for drug susceptibility.

/ Conflicts Of Interest

The authors have no conflicts of interest to declare.

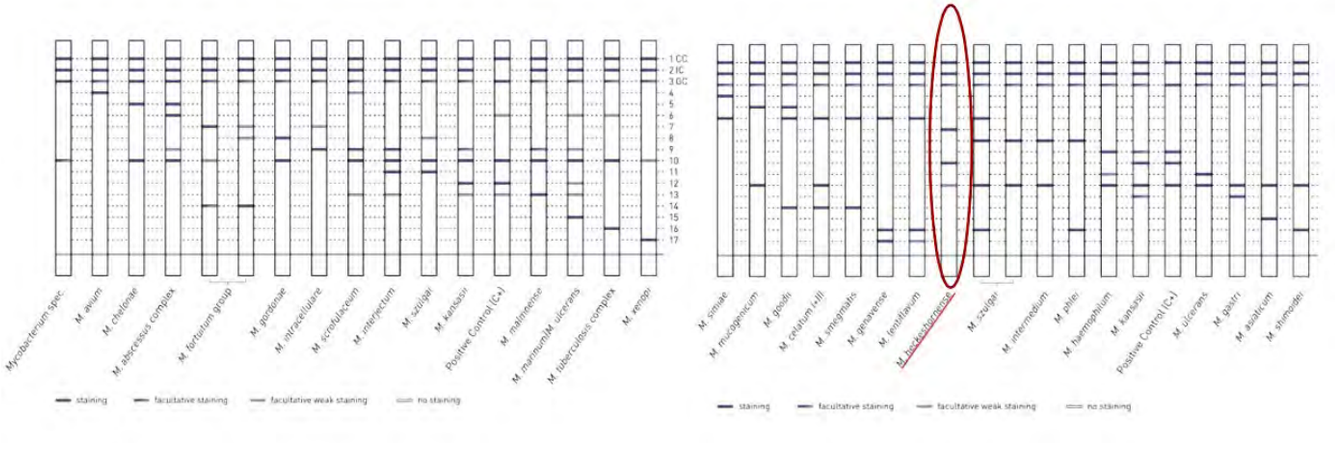

Image 3 – Adapted Image from HainLifescience GmbH - *GenoType Mycobacterium CM and* GenoTypeMycobacterium AS - differentiation of 27 "common mycobacteria" and 19 "additional species"

/ Bibliografia

1. van Hest R, van der Zanden A, Boeree M, Kremer K, Dessens M, Westenend P, et al. *Mycobacterium heckeshornense* infection in an immunocompetent patient and identification by 16S rRNA sequence analysis of culture material and a histopathology tissue specimen. J Clin Microbiol. 2004; 42 (9):4386-9.

2. McBride SJ, Taylor S, Pandey SK, Holland DJ. First case of *Mycobacterium heckeshornense* lymphadenitis.

J Clin Microbiol. 2009 Jan; 47(1):268-70.

3. ElYousfi AL, Leiter JS, Goytan MJ, Robinson DB. *Mycobacterium heckeshornense* lumbar spondylodiscitis in a patient with rheumatoid arthritis receiving etanercept treatment. J Rheumatol. 2009; 36(9):2130-1.

4. Carpenter RJ, Graf PCF. Pott's Disease? AIDS-Associated *Mycobacterium heckeshornense* spinal osteomyelitis and discitis. J Clin Microbiol. 2015; 53(2):716–8.

5. Morimoto K, Kazumi Y, Maeda S, Yoshimori K, Yoshiyama T, Ogata H, et al. *Mycobacterium heckeshornense* lung infection that was diagnosed as *Mycobacterium xenopi* disease by DNA-DNA hybridization (DDH). Intern Med. 2011; 50(11):1251-3.

6. Ahmed R A, Miedzinski LJ, Shandro C. *Mycobacterium heckeshornense* infection in a HIV-infected patient. Emerg Infect Dis. 2010; 16 (11):1801–3.

7. Roth A, Mauch H, Schönfeld N, Loddenkemper R, Reischl U, Naumann L, et al. Description of *Mycobacterium heckeshornense* sp, nov. J Clin Microbiol. 2001; 39(8):3023-4.

8. Van der Werf MJ, Ködmön C, Kataliniy-Jankoviy V, Kummik T, Soini H, Richter E, et al. Inventory study of non-tuberculous mycobacteria in the European Union. BMC Infectious Diseases. 2014; 14(1):62.

9. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, et al: An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007; 175(4):367–416.

10. Thermo Scientific [Internet]. TREK diagnostic systems. [cited 2017 Mar 11]. Available from http://www.trekds.com/products/versatrek/mdst.asp.

11. Hain Lifescience [Internet]. Products. *GenoType Mycobacterium AS VER 1.0;* GenoType *Mycobacterium* CM VER 2.0.[cited 2017 Mar 11]. Available from http://www.hain-lifescience.de/en/products/microbiology/mycobacteria.html.

12. Thermo Scientific [Internet]. TREK diagnostic systems. [cited 2017 Mar 11]. Available from http://www.trekds.com/products/sensititre/c_mycobacterium.asp.