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Case Report

Mycobacterium kumamotonense in the cervical region in an immunocompetent patient, clinical case report in Mexico

Alejandro Hernández-Solis[1], Maribel González-Villa[2], José Ernesto Ramírez-González[2], Yesenia Colin-Muñoz[1], Raúl Cicero-Sabido[1]

[1] Unit of Pulmonology and Surgery of Thorax, General Hospital of Mexico "Dr. Eduardo Liceaga", Mexico City, Mexico
[2] Institute of Epidemiological Diagnosis and Reference (InDRE), Ministry of Health, Mexico City, Mexico

Abstract

Non-tuberculous mycobacterial infection has increased significantly in recent years, especially in emerging countries. We present the case of a 25-year-old male patient, immunocompetent, with cervical lymphadenopathy, identifying Mycobacterium kumamotonense, a rare species in extrapulmonary forms and with a high drug resistance index.

Key words: Non-tuberculous mycobacteria; cervical lymphadenopathy; *Mycobacterium kumamotonense*.

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Introduction

Non-tuberculous mycobacteria (MNT) are those unrelated to the Mycobacterium tuberculosis and Mycobacterium leprae complex. Approximately 170 species known to cause disease in humans are known [1]. These mycobacteria are part of the environment, being identified in soil, water, animals and food products [2]. The pulmonary form is the most frequent in adults, while cervical lymphadenopathies represent the most common extrapulmonary form in children. In patients with HIV / AIDS, disseminated tuberculosis is the main presentation form [1,3]. The incidence and prevalence of pathologies triggered by NTMs have been increasing especially in emerging countries, while in industrialized countries there is a rate that varies from 1 to 1.8 cases per 100,000 inhabitants [4]. The commonly involved agents are *Mycobacterium avium complex*, *Mycobacterium gordonae* and *Mycobacterium xenopi* [5]. Extrapulmonary forms predominate in the female gender with an average age of 50 years, and they are more frequent in skin and soft tissues, followed by disseminated disease and cervical lymphadenopathy [6].

In Mexico there are no epidemiological data or typification of the species involved. There are some case reports in patients with HIV / AIDS in whom *M. avium, M. kansasii, M. gordonae, M. fortuitum* and *M. simiae* were isolated [7]. In previous studies of patients with cervical lymphadenopathy, MNT have been reported only in 6.6% of cases, identifying *M. intracellulare, M. gordonae and M. fortuitum* [3].

The *Mycobacterium terrae* complex (MTC) is the group of mycobacteria that has been linked as the causative agent of bone and joint infections. Among the main species identified in this complex are *Mycobacterium kumamotonense, M. senuense, M. paraterrae, M. strain, M. engbaekii, M. longobardum, M. heraklionense, M. virginiense and M. arupense* [8].

In 2006, a new mycobacterial species, *M. kumamotonense*, was proposed, which has the insertion of 14 nucleotides, which is distinctive of the M. terrae complex in the 16S rRNA gene [9,10].

Here below is the report of a clinical case in which the presence of *M. kumamotonense* in an immunocompetent patient with no history of pulmonary pathology and HIV negative is identified.

Clinical case

We present the case of a 25-year-old male patient, without a history of immunodeficiencies, who goes to a concentration hospital in Mexico City for presenting a 3-month clinical picture characterized by a fever of 38 ° C, loss 5 kg in weight, accompanied by cervical ganglion growth of approximately 3 cm in diameter,

hard, non-painful, asthenia and adynamia. HIV Elisa test was performed and reported negative and imaging studies, in which chest image was found without lung involvement data. The neck CAT (Computed axial tomography) scanned multiple enlarged cervical ganglion chains (Figure 1). Excisional biopsy of lymphadenopathy was performed finding granulomatous reaction and caseous necrosis in the histopathological study (Figure 2). Ziehl-Neelsen staining was performed identifying the presence of acid-alcohol resistant bacilli. In order to confirm the diagnosis of tuberculosis, culture was carried out in a liquid medium for the detection of *M. tuberculosis* with the diagnostic Becton Dickinson kit, Sparks, MD, USA, observing positive growth at 32 days. Subsequently, DNA was extracted with a phenol chloroform method and PCR technique was performed to amplify a 440 bp fragment corresponding to the hsp65 fragment using primers TB11: 5'-ACCAACGATGGTGTCCAT-3 'and TB12: 5'- CTTGTCGAACCGCATACCCT-3. This procedure was performed using ABI PRISM_ 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The phylogenetic analysis was carried out with MEGAv.6.0 software (distance Neighbor Joining method) using the database for MNT species reported by Escamilla-Escobar [11], including the sequences with ID: KX077601 (strain study), KF432567, KF432807 , AB239920, AJ307656, AF547879, GU564405, FJ268582, JN571203, JN571202, KF432509, KT185529obtained from GenBank.

In the results of the protein PCR thermal shock restriction analysis, 65 genes were consistent with those of *Mycobacterium kumamotonense*. The phylogenetic tree was made based on the sequences of the hsp65 gene under the method of the nearest neighbor (Figure 3).

Figure 2. Histological section of cervical lymph node showing chronic granulomatous inflammation with the presence of caseous necrosis with HE staining. 10X.

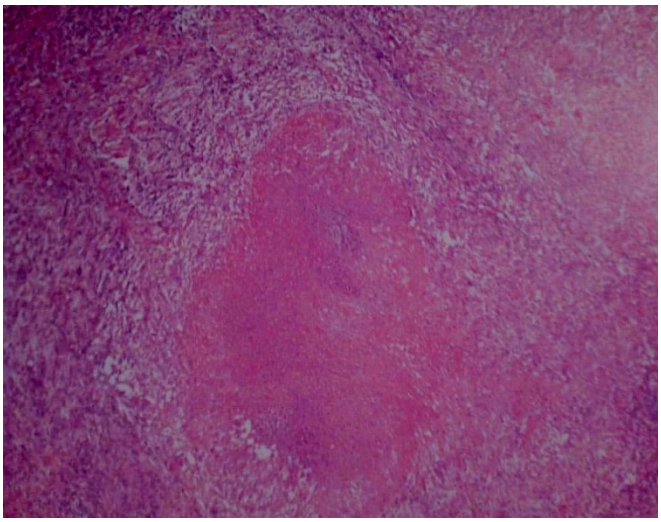
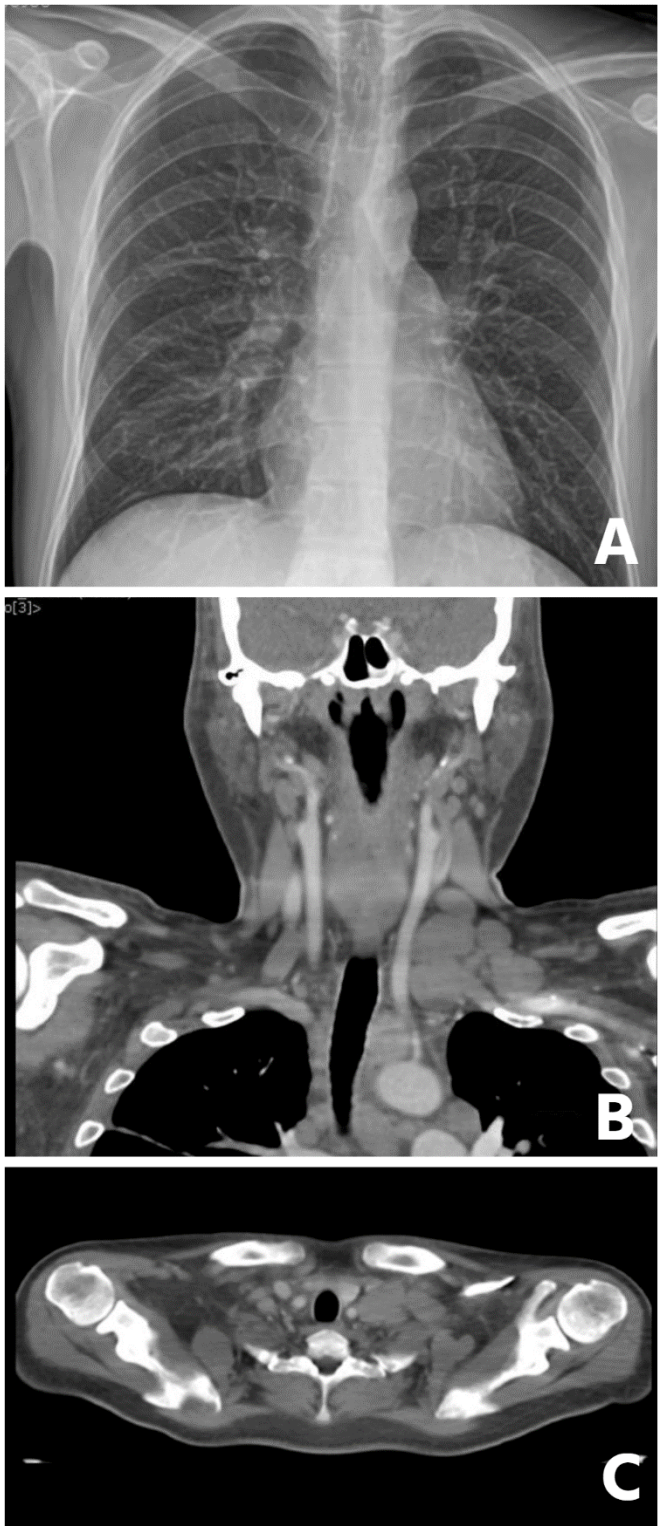


Figure 1. A. Homogeneous calcified image, 1 cm in diameter, in the right hilar region. B. Node conglomerate in left supraclavicular space and paratracheal region which compress the carotid. C. Displaced carotid by node conglomerate.



Once the diagnosis was confirmed, the patient received treatment with Isoniazide, Rifampicin and Etambutol. After three months of treatment, a favorable evolution was observed, with the disappearance of the febrile symptoms and a marked decrease in the volume of the cervical nodes. There were no recurrences after one year of follow-up.

Discussion

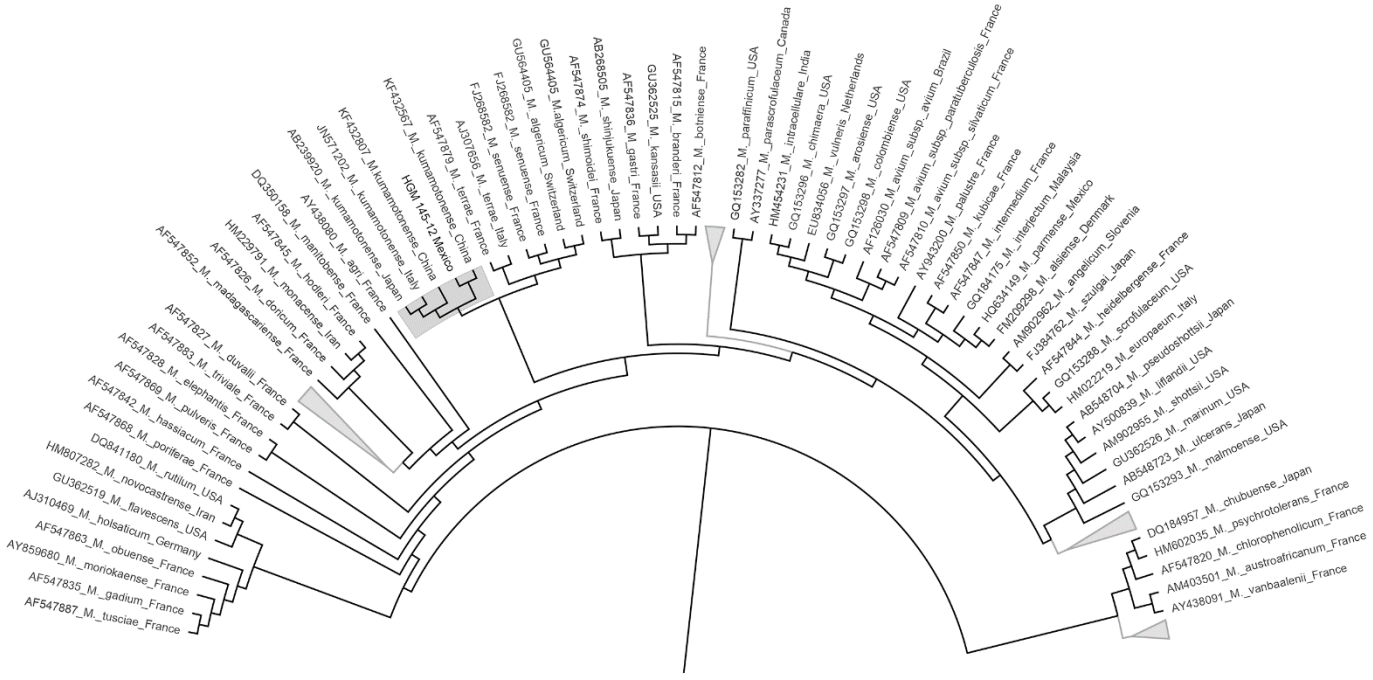
Mycobacterium kumamotonense is a slow-growing mycobacterium, which belongs to the *M. terrae complex*. Data on the incidence of this species are limited globally. It was first isolated in sputum material from an immunocompetent patient . The condition of subjects carrying HIV by MNT occurs after the CD4 count is < 200 cells / mm^3. In the opposite case, interferon-specific (IFN) γ and interleukin (IL) -12 mutations have been associated in non-HIV-bearing subjects. The incidence of MNT disease is high in patients suffering from immunosuppression, connective tissue diseases, diabetes mellitus or using steroid corticosteroids. Mortality is significantly increased in patients older than 65 years, of the female gender and co-infection with HIV / AIDS [12,13].

In the literature, cases reported about the human condition of *M. kumamotonense* are rare, so the clinical characteristics are still very variable. Clinically, these cases are characterized by presenting pulmonary symptoms and in the CAT scan nodular images, caverns and bronchiectasis are shown. Unlike what was found in the literature, our patient is male, under 35 years old, with extrapulmonary condition, without relevant risk factors and has no respiratory compromise [6,14].

The clinical picture of lymphadenopathy is similar between different species of mycobacteria. These are presented as persistent local inflammation, with few general symptoms, indurated chains of gradual growth and rarely form fistulas, such as those observed in our patient. In cervical lymphadenopathies, surgical excision is associated with high cure rates, in combination with antimycobacterial therapy. In our case, we had an adequate response to treatment without the presence of disease recurrences, possibly because the patient was not immunocompromised and did not present a multidrug resistant drug strain. In the literature, antimicrobial therapy with macrolides is recommended and preferably drug sensitivity studies to initiate specific treatment to avoid a chronic clinical picture and relapse [13,14,15].

The identification of MNTs is of the utmost importance to establish an adequate treatment. Currently, various methods for such identification have been described, however, the results remain uncertain due to the limited experience with this type of mycobacteria. Both, thin layer chromatography and gas-liquid chromatography have been considered for the identification of various species of mycobacteria because their cell wall has a rich lipid component. On

Figure 3. Phylogenetic tree constructed with hsp65 gene sequences. Strain HGM_145-12 (black framed) was identified as M. kumamotonense due to its phylogenetic relationship with this cluster.



the other hand, mass spectrometry has also been used, although one of its main limitations is the lack of standardization in the preparation of samples for the specific identification of these species [10].

The most commonly used methods are probes, which identify the most common subtypes of mycobacteria. The identification of mycobacteria by the PCR-RFLP (Restriction fragment length polymorphism) method of hsp65 has been shown to have advantages over conventional methods for being fast, lower cost and highly specific with results in 48 hrs. It has been reported that the hsp65 gene is present in all mycobacteria, is more variable than the 16S rRNA gene sequence and is therefore potentially useful for the identification of genetically related species. In our case, a culture medium specific for *M. tuberculosis* was used for the identification of *M. kumamotonense*. Once identified, the hsp65 gene was amplified, which showed 100% identity with *M. kumamotonense*, so, within the various methods proposed, we agree with what is reported in the literature in which PCR is the most accurate method and accessible for the correct identification of the multiple subtypes of mycobacteria [16].

Although NTMs are usually related to nosocomial infections, there is little evidence of the person-to-person spread of this type of pathogens and the range of diseases that can lead is wide, so the rapid and accurate identification of mycobacteria to the Species level is essential to facilitate early treatment of mycobacteriosis [17,18].

Conclusions

It is important to note that health personnel do not suspect the presence of MNT in immunocompetent patients with extrapulmonary disease and much less to the isolation of *M. kumamotenense*, a species little studied and unfortunately resistant to first-line medications, causing a chronic disease in the most cases.

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Corresponding author
Alejandro Hernández Solis
Unit of Pulmonology and Surgery of Thorax,
General Hospital of Mexico "Dr. Eduardo Liceaga"
Dr. Balmis 14. Col. Doctores. México D.F. CP 06726
Tel: +5215568572559
E-mail. drhernandezsolis@yahoo.com.mx

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