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CASE REPORT

First isolation of *Tropheryma whipplei* from bronchoalveolar fluid and clinical implications

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Summary A patient presented diffuse pulmonary parenchymal micronodules. *Tropheryma whipplei* was detected in the saliva, a bronchial biopsy and bronchoalveolar fluid. PAS staining, immunohistochemistry and PCR for *T. whipplei* were negative in the duodenal biopsies. *T. whipplei* was isolated from the bronchoalveolar fluid, reinforcing its role as a respiratory pathogen.

Introduction

Tropheryma whipplei is the bacterium associated with the disease described by George H. Whipple in 1907, consequently called Whipple's disease.[1] This bacterium was first identified in 1991 using molecular assays including nucleotide sequencing and amplification of a segment of the bacterial 16S ribosomal DNA present in a small-bowel biopsy specimen taken from a patient with classic Whipple's disease.[1] However, in 2000, the bacterium was isolated for the first time from a patient who did not have classic Whipple's disease; the first established strain of *T. whipplei* was isolated from a cardiac valve of a patient with localized endocarditis due to *T. whipplei*.[1] Since then, the bacterium has been cultured several times from various specimens from patients with Whipple's disease, including specimens from duodenal biopsies, stools samples, cerebrospinal fluid, lymph node biopsies, skeletal muscular biopsies, skin biopsies, and articular fluids.[2,3]

In addition, the development and the improvement of molecular tools have allowed the detection of *T. whipplei* DNA in several specimens from chronic carriers who exhibit high levels of antibodies against *T. whipplei* but do not have Whipple's disease.[4] In particular, *T. whipplei* DNA has been detected in stool and saliva specimens.[5,6] The

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prevalence of chronic carriage depends mainly on the geographic distribution and on occupation.[5,6] In the French adult general population, this prevalence is approximately 2—4%, whereas it is approximately 12% among French sewer workers. In rural Senegal, in Africa, *T. whipplei* DNA has been detected in up to 44% of children from 2 to 10 years old.[5,6] The carriage of *T. whipplei* DNA in the saliva is less frequently observed, being present in 0.5% of the French adult general population and 2.5% of French sewer workers.[5,6]

More recently, the role of *T. whipplei* as a pathogenic agent has been suspected in highly varied and common clinical manifestations, such as gastroenteritis among children in Africa and in France, bacteremia in patients with fever and without malaria in Africa, and in patients with pneumonia.[6—8] Indeed, *T. whipplei* DNA has been previously found several times from respiratory specimens associated with pneumonia.[8,9] These data have been obtained by two different teams. In the United States of America, *T. whipplei* DNA was detected in the bronchoalveolar lavage fluid of a child with pneumonia who was included as a control in a metagenomic study for patients with cystic fibrosis, and in France, *T. whipplei* DNA was detected in the bronchoalveolar lavage fluid of adults with pneumonia hospitalized in the intensive care unit.[8,9]

However, to date, *T. whipplei* has been isolated in culture only one time from a healthy carrier, in that case from the saliva,[10] and we report herein, for the first time, the culture of *T. whipplei* from a bronchoalveolar fluid sample.

Patients, methods and findings

Case report

In August 2010, a 70-year-old woman was admitted to the hospital as she developed for 3 weeks nocturnal sweats and fever as well as dyspnea, myalgia and arthralgia. Her medical history included a cholecystectomy performed in 1987 and scoliosis as well as a history of diarrhea, 3 months before, which started just after a trip to Morocco. A chest X-ray revealed bilateral and diffuse parenchymal micronodules and mediastinal adenopathies. Thoracic-abdominal-pelvic computed tomography confirmed the presence of the mediastinal and hilar lymphadenopathies and diffuse bilateral micronodular involvement without other abnormalities (Fig. 1). No blood cell count abnormalities were observed. The C-reactive protein level was also normal. A bronchoalveolar fluid sample was first inoculated for standard culture onto Columbia CNA (colistin and naladixic acid) with 5% sheep blood and chocolate agar (bio-Mérieux, Marcy L'Etoile, France), incubated at 37 °C in a 5% CO_2 atmosphere and onto Mc Conkey agar (bio-Mérieux) incubated at 37 °C for 48 h. The bronchoalveolar fluid sample was also inoculated onto BCYE (buffered charcoal-yeast extract) agar medium (bio-Mérieux) incubated at 37 °C for 7 days, onto homemade Hayflick media incubated at 37 °C in a 5% CO_2 atmosphere for 21 days and onto BACTEC MGIT 960 System (Becton—Dickinson Diagnostics, Loveton Circle Sparks, MD, USA) for 1 month to look for respectively, *Legionella* spp, *Mycoplasma pneumoniae* and *Mycobacteria* spp. All the inoculated media were sterile.

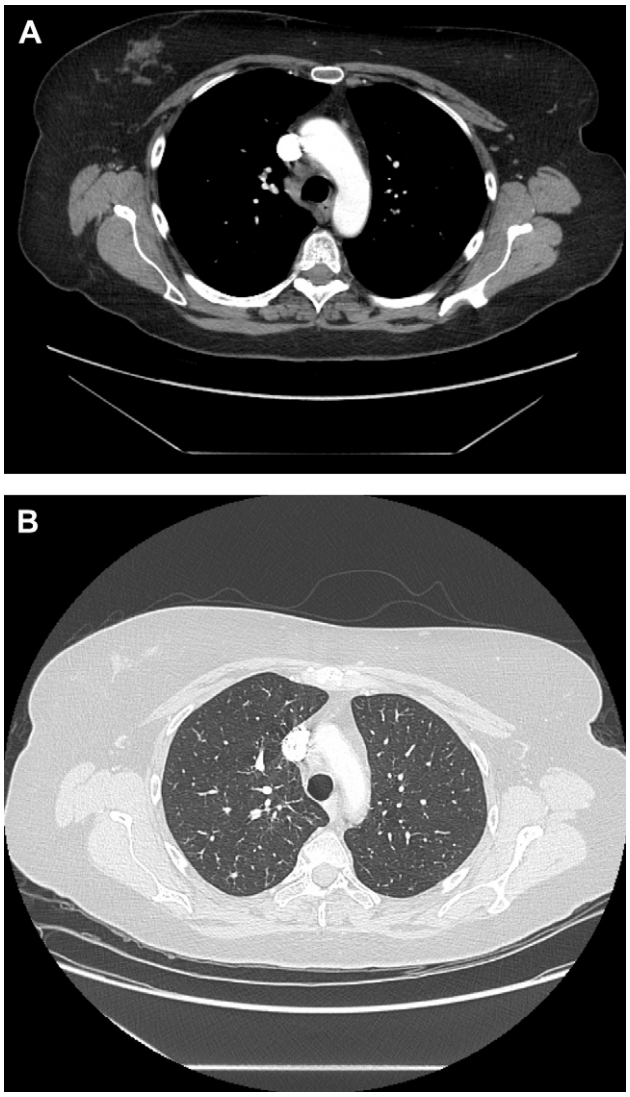

Figure 1 Enhanced thoracic CT-scan. A: Mediastinal and hilar lymphadenopathies. The largest mediastinal lymphadenopathy involvement was 16 × 14 mm in the Barety's space. B: Diffuse bilateral micronodular involvement.

In parallel, serology assays in order to detect microbial agents of interstitial pneumonia, including *M. pneumoniae*, *Legionella pneumophila*, *Chlamydophila pneumoniae*, *Chlamydophila psitacii*, and *Coxiella burnetii* were performed. All the assays were negative.

Because of mediastinal and hilar lymphadenopathies, arthralgias, recent episode of diarrhea, the role of *T. whipplei* was suspected. *T. whipplei* quantitative real-time PCR (qPCR) targeting repeated sequences was performed as previously reported.[11] The quality of DNA handling and the extraction of the specimens were also carefully checked by qPCR targeting the house-keeping gene coding for β-actin. Using these molecular assays, a saliva sample, a lung biopsy specimen as well as the bronchoalveolar fluid sample were found to be positive for *T. whipplei*, whereas a blood specimen, cerebrospinal fluid and small-bowel biopsy specimens were negative. *T. whipplei* qPCR performed on a stool specimen was not interpretable because the