Cord factor producer *Mycobacterium abscessus* subsp. *bolletii* in asymptomatic immunocompetent host sputa samples

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We report a case of *Mycobacterium abscessus* subsp. *bolletii* colonization in upper respiratory tract of an immunocompetent patient, who was misdiagnosed as tuberculosis by Acid Fast Bacilli (AFB) and cord factor formation observed directly from the sputa culture in liquid medium. This fact reflected a significant impact on the individual case’s life and showed the importance to identify the mycobacteria isolated from clinical sample at species level, and to determine the true implication of nontuberculous mycobacteria (NTM) detected in clinical samples.

**Keywords:** Nontuberculous mycobacteria. Tuberculosis. Diagnosis. Microscopy. Molecular diagnosis.

INTRODUCTION

Microscopic cord factor aggregation is a phenotypic characteristic of *Mycobacterium tuberculosis*, firstly reported by Robert Koch in 1882, and associated with virulence since 1947 by Middlebrook and colleagues (1974). It is composed by tight bundles formed by parallel alignments of bacilli, which are historically considered a distinct trait, and used as a practical criterion for rapid and presumptive identification of *M. tuberculosis* isolates in clinical laboratories (Carter, Ratkiewicz, 1998).

The cord factor aggregation has been related to intracellular replication and granuloma formation by *M. tuberculosis* (Yamagami et al., 2001). In other mycobacteria, the occurrence of cord factor aggregation was demonstrated in some nontuberculous mycobacteria (NTM) distantly related to *M. tuberculosis*, which includes *Mycobacterium marinum* and *Mycobacterium Abscessus* Complex (Julián et al., 2010). Likewise, cord producers *M. abscessus* Complex tend to harbor drug resistance (Rüger et al., 2014) with severe lung disease (Sanguinetti et al., 2001).

NTM form a large group within the *Mycobacterium* spp., and show wide environmental distribution. More than 100 species are found in soil, potable water, food, and animals. Inside the NTM group, the rapidly growing mycobacteria (RGM), which are able to produce mature colonies on agar plates within 5 to 7 days, have been considered important human pathogens in the last decades (Pang et al., 2015).

The pathogenicity of NTM species, whether in man or in animals, ranges from an innocuous colonization to a wide kind of human diseases, mainly pulmonary and soft tissue infections. The detection of NTM, in clinical specimens, can have no clinical significance. The determination of their clinical significance in such specimens is sometimes not easy to interpret, and requires specific criteria to make the distinction between...
colonization and disease (mycobacteriosis). Also, the identification of the NTM, at species level, is laborious and sometimes inconclusive.

The increase of NTM caused diseases and the laboratory challenges for presumptive differentiation of NTM bacillus motivated us to write this case report. This case study aimed to alert the microbiologist about the importance of the correct identification of the Mycobacterium species and its impact for the health and life of the patients.

CASE PRESENTATION

Due to traveling requirements, an asymptomatic 23-year-old female was assisted by a primary care physician. A chest X-ray done at the time did not reveal thoracic alterations. However, the acid-fast staining microscopy of expectorated sputa showed the presence of acid-fast bacilli (AFB) in two independent samples (+ and ++ in the acid-fast staining microscopy by Ziehl Neelsen, Z-N). At that time, tuberculosis therapy was started, according to the World Health Organization recommendation (WHO, 2016; Brasil, 2011). In parallel, two new sputa samples cultures were required. The cultures of samples were carried out on BD BACTEC MGIT System (Mycobacteria Growth Indicator Tube) (BD Difco™ BBL™, USA). After 5 days of cultures incubation, it was observed AFB growth, which were subcultured in Lowenstein-Jensen medium. After the same time of incubation at 35 ºC in normal atmosphere, smooth white colonies were observed.

Until mycobacterial identification, a gradually smooth to rough reversion in the colony morphotype was observed in bacillus subcultures in Lowenstein-Jensen medium. These colonies morphology changes occurred by the predominance of smooth white colonies on the first culture, and the progressive predominance of rough colonies in the last ones. The Z-N from the Middlebrook 7H9 medium, added of 0.2 % glycerol (SIGMA – Aldrich St Louis Mo, USA) and supplemented with OADC(BD Difco™ BBL™, USA), showed acid-fast cord factor aggregation after subsequent culture. Identification at the species level was performed by PCR-restriction fragment length polymorphism analysis (PRA) of a 441 bp hsp65 fragment. Interpretations of PRA-hsp65 patterns in PRA site were characteristic of Mycobacterium abscessus subsp. bolletii (BstEII, 235/210/0; HaeIII, 200/70/60) (http://app.chuv.ch/prasite/index.html).

As no clinical symptoms and lung impairment was correlated with NTM disease, and no immunocompromised background such as HIV/AIDS or a primary immunodeficiency was detected in the patient, the tuberculosis treatment was suspended. No treatment for NTM was introduced and the patient has been clinically followed by the clinician.

The patient signed the informed consent form for the publication of this clinical case, and reported that the main impact on her personal life was the interruption of an international scholarship, scheduled at that time, due to the misdiagnosis of tuberculosis. In addition, as a consequence of the anti-tuberculosis therapy, the patient suffered from adverse effects. The study was approved by the Ethics Committee (COPEP) of the State University of Maringá, Brazil, according to Portaria N° 004/ 2013, under opinion nº 1.937.

DISCUSSION

This report raises the question of preliminary identification of M. tuberculosis by the presence of cord factor aggregation, which is widely used in low income clinical laboratories. This come to reinforce the need for the correct and rapid identification of the acid fast bacilli (AFB), detected in clinical samples, at species level.

All mycobacteria species have an unusual cell wall structure, in which outside the plasma membrane, a non-fluid hydrophobic fatty acid layer supports a fluid monolayer rich in glycolipids as trehalose 6,6-dimycolate – TDM (cord factor). This cell wall composition plays a role in the prevention of bacterial desiccation, and TDM seems to be an important antigen for the immune modulation in M. tuberculosis infections (Harland et al., 2008).

The smooth to rough colony morphotype reversion, observed in M. abscessus subsp. bolletii isolated from the sputa samples in this case, is a recognized change that occurs in NTM cell wall composition, which leads to increase in the bacilli virulence and survival.
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into macrophages (Julián et al., 2010). This colony morphology variation occurs due to a defect in the cell wall glycopeptidolipid (GPL) production that has been observed in *M. abscessus* Complex, including *M. abscessus* subsp. *bolletii* (Howard et al., 2006; Medjahed, Reyrat, 2009). Such change of *M. abscessus* colony phenotype was recently, related to mutations in the *gpl* gene, which codify GPL, leading to a defective GPL production, as demonstrated in *M. abscessus*, as well as in *M. abscessus* subsp. *bolletii* clinical isolates (Kim et al., 2013).

According to Howard et al. (2006) the location of GPL in the outer most portion of the cell wall in *M. abscessus* smooth phenotype colony can prevent the interaction of TDM molecules from contiguous bacteria necessary for cording formation. This explains the observation of cording formation in *M. abscessus* subsp. *bolletii* detected after subcultures of sputa from the individual case in this study.

The cording factor formation in *M. abscessus* Complex was observed previously, which involves glycolipid trehalose-6,6'-dimycolate (TDM), and is associated with the virulence of bacterial variants to persist and cause invasive disease (Howard et al., 2006).

We can argue here that the false tuberculosis diagnosis was first indicated by AFB smear (++). Also, it is important to emphasize that the *M. abscessus* subsp. *bolletii* detection does not prompt to a disease, which can create diagnostic errors and lead to unnecessary and wrong treatment, as occurred with the patient case. The determination of clinical significance of NTM isolated in sputum as a disease-causing or as part of transient microbiota is not always easy, and requires specific criteria (Li et al., 2017). It is important to emphasize that, in the present case, the *M. abscessus* subsp. *bolletii* was isolated in two primary sputa samples and in another requested by the laboratory for case confirmation.

Diseases caused by NTM are associated with the natural resistance to many antibacterial drugs, which leads to disappointing clinical results from currently available therapy. This notorious situation is not different with *M. abscessus* Complex, as it is one of the most chemotherapeutic resistant NTMs, responsible for poor treatment outcomes (van Ingen, Kujiper, 2014).

The present case report raises the question of how important it is to carry out diagnosis assays for identification of mycobacteria at species level, in clinical specimens, as soon as possible, to introduce the correct treatment and confirm the NTM susceptibility by drug susceptibility testing.

**CONCLUSION**

The present study draws attention to the importance of identifying the mycobacteria species in clinical sample and determine the true implication of NTM detected as the causative agent of the disease. Furthermore, the treatment for tuberculosis differs from those for diseases caused by NTM. A misdiagnosis can have serious consequences for the mental and social health of the patient.

**CONFLICT OF INTERESTS**

None to declare

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