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CLINICAL CASES

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Identificación de Mycobacterium fortuitum por espectrometría de masas MALDI-TOF en hemocultivo

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ABSTRACT

Non-tuberculous mycobacteria (NTM) belong to the genus Mycobacterium which comprises several species such as those of the *Mycobacterium fortuitum* group. Infections in post-surgical situations, as well as in hemodialysis patients, especially those with immunocompromising, are not uncommon. In spite of the diverse sites in which such microorganism can be isolated, the cases in which systemic infection occurs are not common. The aim of this study was to present a case report of *M. fortuitum* in blood culture. Identification was performed by MALDI-TOF mass spectrometry and Real-Time PCR-RFLP confirmation.

Keywords: *Mycobacterium infections, Nontuberculous; Mycobacterium fortuitum; Spectrometry, Mass, Matrix-Assisted Laser Desorption-Ionization; Polymerase Chain Reaction.*

INTRODUCTION

The Mycobacterium genus is composed of several species, with emphasis on non-tuberculous mycobacteria (NTM) that form a group of more than 140 species identified through phenotypic tests (growth time, pigment production or not, biochemical tests, growth in presence of chemical inhibitors) and molecular tests.[1]

Among the NTMs is the *Mycobacterium fortuitum* group, which is known to be associated with reports of lung diseases in humans, as well as being responsible for the majority (60-80%) of cases of post-surgical infections, and catheter-related infections caused by NTM.[2]

M. fortuitum is known to be a ubiquitous environmental organism that has been isolated from soil, water and many other sources.[2] In spite of being a rare pathogen, due to the extensive application of immunosuppressant, there has been an increase in its incidence, with the main clinical manifestations being pulmonary disease, lymphadenitis, cutaneous disease, and disseminated disease.[3] There are documented cases of infection from peritoneal dialysis, associated with peritonitis, as well as septic arthritis and corneal keratitis.[4] Although several clinical manifestations have been reported, septicemia is still rarely reported.[5]

Usually, infections do not occur in healthy patients, and there is a correlation with surgical and post-traumatic wounds and immunocompromised patients.[6] The association with disseminated lesions in patients on dialysis has also been reported.[7]

In general, *M. fortuitum* presents good susceptibility to several antimicrobials, such as amikacin, ciprofloxacin, ofloxacin, Sulfonamides, and imipenem.[4]

Thus, because it constitutes a relevant infection and is not usually correlated with a systemic course, the aim of this

study was to present a case report of *M. fortuitum* in blood culture, through MALDI-TOF mass spectrometry, a fast, good assertiveness, and low-cost methodology.

CASE REPORT

A 55-year-old female patient, a resident of a municipality in the country of the state of Minas Gerais, with chronic renal failure and periodically on hemodialysis. After a strong pyrogenic reaction, the patient was referred to ICU and started therapy with cefazolin and gentamicin.

Three blood culture sample was collected and incubated in BacT/ALERT® system (bioMérieux®, Brazil). After 30 hours of incubation, the automated system indicated that one blood culture vial presented growth. An aliquot of the blood vial was plated onto a BAP (bioMérieux®), which was incubated at 37 °C, presenting bacterial growth after 48 hours (Figure 1). The colonies were submitted to VITEK® 2 Compact (bioMérieux®) automated system, however, the microorganism was not identified.

This was a case report study. The identification was realized by MALDI-TOF MS (VITEK® MS - bioMérieux®) and patient data were obtained and discussed among the professionals involved. The identification score was provided by middleware MYLA (bioMérieux®, France). For MALDI-TOF MS analysis, protein extracts were prepared from bacterial isolates grown on a BAP (bioMérieux®) and suspended in 10% of formic acid (bioMérieux®). One microliter of the mixture was spotted onto a polished steel target plate (bioMérieux®) and air-dried. Then 1 µl of a saturated solution of α-cyano-4-hydroxycinnamic acid in acetonitrile 50% and trifluoroacetic acid 2.5% (bioMérieux®) was added and the mixture was allowed to cocrystallize at room temperature.

The colonies were assessed using the VITEK® MS (bioMérieux®) automated system, which identified them as *M. fortuitum* (99% of probability), as depicted by a characteristic protein fingerprint (Figure 2). The identification was further confirmed by PCR followed by an enzymatic restriction, called PCR-RFLP, evidencing the bands. From this banding profile (Figure 3) the specie was identified using an international database (http://app.chuv.ch/prasite/index.html). The primers used were TB11: ACCAACGATGGTGTGTCCAT and TB12: CTTGTCGAACCGCATACCCT coding for a toxic shock protein (hsp)

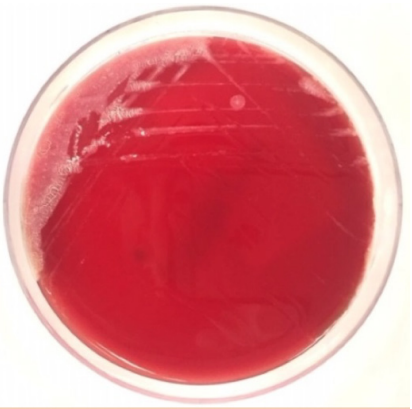

Figure 1. Colonies of *M. fortuitum* on a blood agar plate.

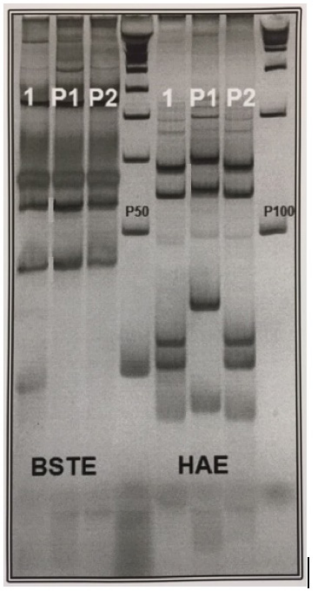

Figure 3. Representative PCR-RFLP result for the clinical isolate amplified, evidencing the use of the enzymes BSTE and HAE.

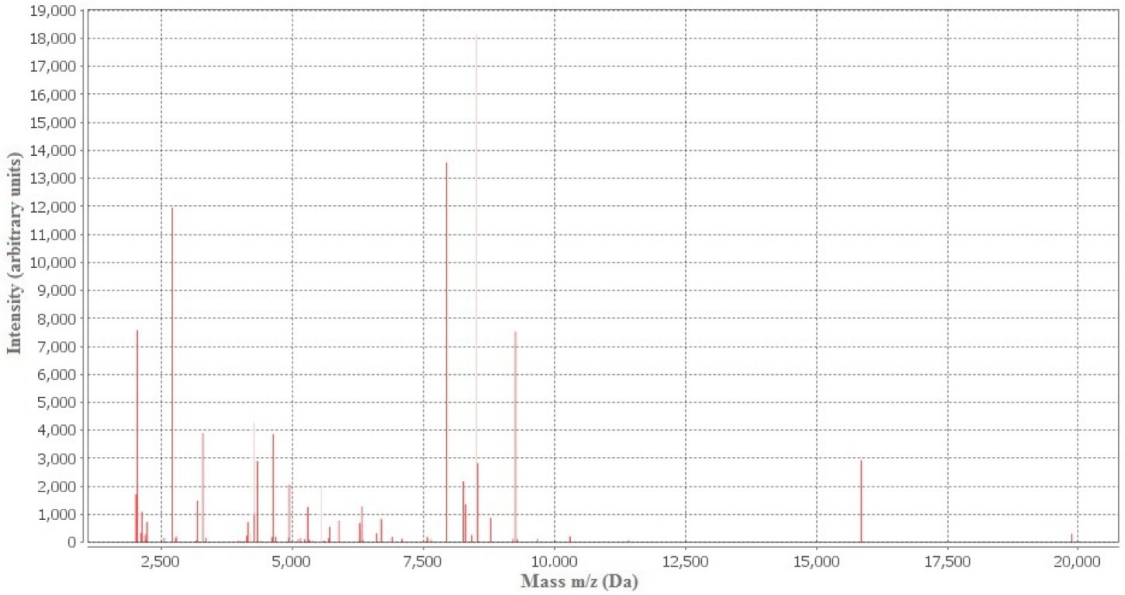

Figure 2. MALDI-TOF MS spectrum of *M. fortuitum*.

recommended for identification of microorganisms of the genus.

The result was immediately reported to the clinician in charge who changed the treatment to amikacin.

DISCUSSIONS

Infection with *M. fortuitum* is more frequent in patients with immunosuppressive therapy, although there are reports of infection by *M. fortuitum* in immunocompetent patients, making their pathogenicity dependent on the opportunity for transmission and susceptibility of the host.[8]

Usually, species of the *M. fortuitum* group are not involved in cases of disseminated disease when compared to other species of pathogenic fast-growing mycobacteria, especially *M. chelonae* and *M. abscessus*[5].

On the other hand, there are reports of NTM infections in hemodialysis patients, some of which have evolved into a widely disseminated disease, demonstrating the virulence potential of the disease.[9]

The infection reported in the present study may be justified by the characteristics of the patient in question. Due to chronic renal insufficiency and periodic hemodialysis sessions, it is expected that the patient has a certain immunological weakness and, therefore, greater risk of contamination, given that the venous accesses can act as a gateway to the microorganism. In addition, the conditions of the dialyzers used should be taken into account, since NTM infections have been reported in situations where the use of disinfectants has been inadequate.[10]

The initial treatment did not present a good response, however, due to the rapid identification of the microorganism and change of the treatment to amikacin, there was a good prognosis. This is in accordance with what has already been demonstrated in other studies which have reported the susceptibility of the *M. fortuitum* group to this antimicrobial at rates ranging from 86.6%[11] to 100%.[4]

Although antimicrobial susceptibility testing has not been performed, it is worth noting the importance of both microbiological tests and drug susceptibility investigations, avoiding unsuccessful and potentially harmful treatments, given that NTMs present higher incidence are resistant to the main drugs used.[12]

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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