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Identification of *Cryptococcus neoformans* by MALDI-TOF mass spectrometry in blood culture

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ABSTRACT

This is a report of isolation of *Cryptococcus neoformans* from blood culture. Identification was conducted by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry. The relevance of this report is related to the site affected, the severity of the infection, and the importance of correct and rapid identification of the causative pathogen for a successful treatment and for reducing the risk of morbidity and mortality.

Keywords: *Cryptococcus neoformans; blood culture; spectrometry; mass; Matrix-Assisted Laser Desorption-Ionization*

Cryptococcosis is a systemic mycosis caused by the inhalation of fungi of the *Cryptococcus neoformans* species complex and commonly observed in immunocompromised individuals[1]. Although the nomenclature and number of the *Cryptococcus* species is a matter of debate, it was recently established that the genus contains at least seven pathogenic species[2].

The *Cryptococcus neoformans* species complex consists of ubiquitary microorganisms which cause opportunistic mycosis in immunocompromised hosts. Other species, such as *Cryptococcus gattii*, may cause primary mycosis in apparently immunocompetent hosts and are endemic in tropical and subtropical areas. Nevertheless, both species can lead to meningoencephalitis with serious or even fatal outcomes, which may include evident pulmonary lesion, fungemia and secondary foci of infection in skin, bones, kidneys, and other organs.

It is known that *Cryptococcus* spp infection is a major cause of mortality in developing countries and represents one of the most important fungal infections in Brazil, remaining a challenge in medical practice.

In this context, the rapid and correct identification of *Cryptococcus* species is of paramount importance, and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) is an excellent alternative to conventional methods. It is an innovative method that evaluates the generation of protein fingerprint of microbial cells after exposure to a laser and has been successfully applied for the rapid identification of bacteria and filamentous fungi[3,4]. With regard to pathogenic yeasts, the simple and fast protein extraction step is required to obtain reliable results[5,6].

The aim of this study is to present a case report of *C. neoformans* in blood culture.

METHODS

This was a descriptive study with case report. The identification of the microorganism was performed by MALDI-TOF MS and patient data were obtained after discussion among the professionals involved. For MALDI-TOF MS analysis, protein extracts were prepared from cryptococcal isolates grown on a blood agar plate (BAP) (Biomerieux®, Brazil) for 24 h at 36 °C and suspended in 10% of formic acid (Biomerieux®, Brazil). One microliter of the mixture was spotted onto a polished steel target plate (Biomerieux®, Brazil) and air dried. After air dehydration, 1 µl of a saturated solution of α-cyano-4-hydroxycinnamic

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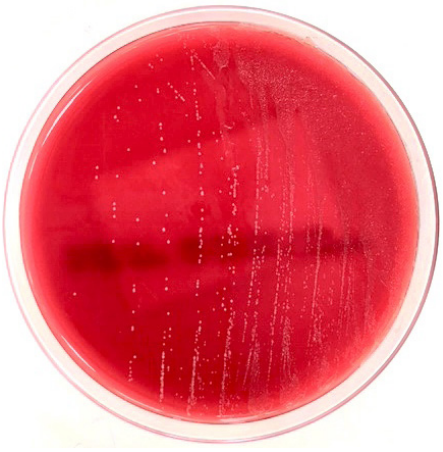

Figure 1: Colonies of *C. neoformans* on a blood agar plate.

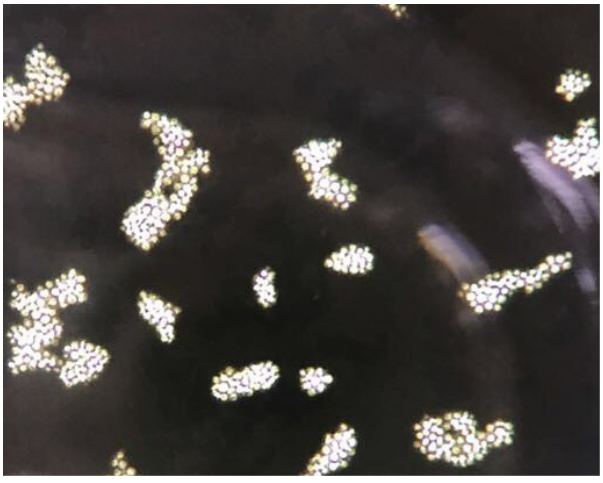

Figure 2: Capsules of *C. neoformans* on a wet mount microscopy slide stained with India ink (40x objective lens).

acid in acetonitrile 50% and trifluoroacetic acid 2.5% (Biomerieux®, Brazil) was added and the mixture was allowed to cocrystallize at room temperature.

RESULTS AND DISCUSSION

A 66-year-old female patient with pneumonia, pulmonary fibrosis, and collagenosis was admitted to the intensive care unit (ICU). Initial antibiotic therapy included clarithromycin, vancomycin and piperacillin-tazobactam. Two blood samples were collected and incubated in the BacT/ALERT® system (BioMerieux). After approximately 4 hours of incubation, the automated system indicated that both blood culture vials presented growth. An aliquot of the blood vials was plated onto a BAP (Biomerieux®, Brazil), which was incubated at 37 °C. The BAP presented growth of small gray mucoid colonies (Figure 1). Initially, the colonies were visualized on a wet mount microscope slide stained with India ink and it was possible to observe budding yeast cells with a characteristic polysaccharide capsule (Figure 2). The colonies were assessed using the VITEK®2 Compact automated system, which identified them as *C. neoformans* (99% of probability). The identification was further confirmed by MALDIT-TOF MS (VITEK® MS), as depicted by a characteristic protein fingerprint of *C. neoformans* (Figure 3). An antifungigram was performed and indicated that the yeast was susceptible to amphotericin B (minimum inhibitory concentration [MIC] = 1.0 µl/mL). Due to the identified microorganism, only amphotericin B was tested. Subsequently, two new blood culture samples were collected, and no positivity was observed.

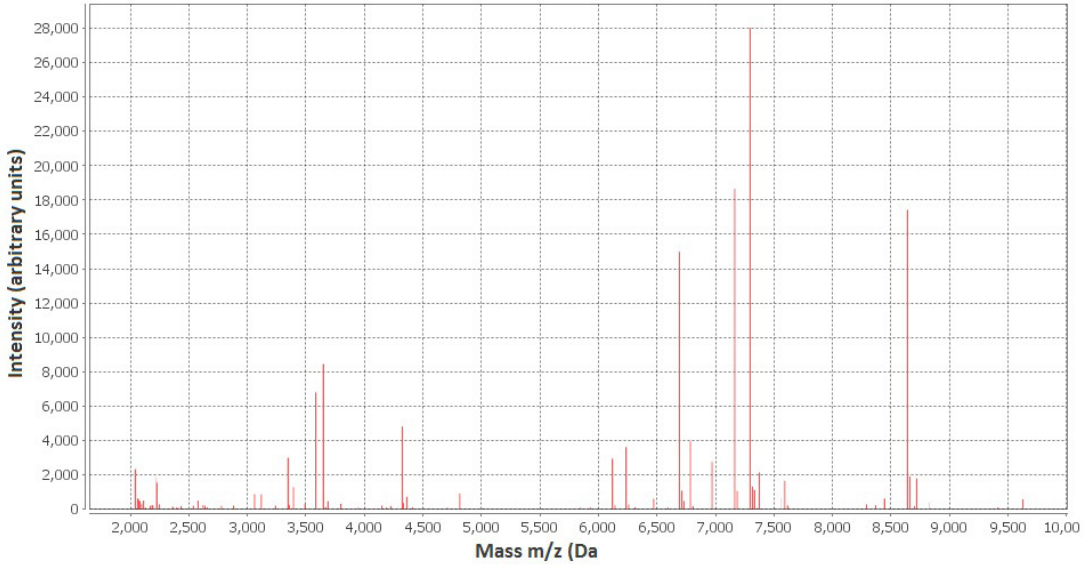

Figure 3: MALDI-TOF MS spectrum of *C. neoformans.*

CONCLUSION

Cryptococcosis is one of the most important systemic mycoses. The clinical presentation of patients should always be evaluated and the presence of other concomitant pathologies should be investigated, since the higher mortality in immunocompromised patients is notorious. Amphotericin B, either isolated or associated with 5-fluorocytosine, and fluconazole are considered the antifungal of choice for treatment[7].

In the present case, there was a direct and clear communication between the laboratories involved and the physician responsible for the patient. There was adequate and timely use of amphotericin B, enabling the improvement of the clinical picture and the discharge from the ICU. There was no fever and C-reactive protein and leukocyte cell counts were normal.

Employing MALDI-TOF MS for analysis was adequate, rapid and assertive. No additional tests were performed to confirm the species, taking into account that the database used is sufficient for the correct identification of the main species of *Cryptococcus*. Previous studies proved the efficacy of MALDI-TOF MS for the correct differentiation between *Cryptococcus neoformans* and *Cryptococcus gattii*[8].

Conflicts of Interest

The authors declare no conflicts of interest.

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