International Journal of Research in Medical Sciences
Belda W et al. Int J Res Med Sci. 2023 Oct;11(10):3843-3848
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20233044

Case Report

Mycetoma caused by *Trichosporon asteroids*-report of the first case

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Received: 31 July 2023
Revised: xx May 2023
Accepted: 02 September 2023

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ABSTRACT

Mycetoma is a neglected disease that affects mainly the skin, but can progress to deep tissues and structures such as muscles and bones. Mycetoma can be caused by some bacterial (actinomycetoma) or fungal species (eumycetoma); furthermore, eumycetoma is estimated to account for 40% of all cases of mycetomas. Regardless of etiology, human infections occur after accidental implantation of etiological agents through the skin. In the present work, an immunocompetent patient without systemic comorbidity is reported to have exhibited a progressive increase of the left foot with multiple fistulas in the dorsum in the last 15 years, which emerged as a purulent secretion in the presence of yellowish grains. The patient reported that during a trip she suffered injuries in the affected foot. Histopathological study showed the presence of fungal grains, and the culture of skin fragments allowed the identification of fungal colonies exhibiting a dry, cream-coloured cerebriform morphology with a radiated peripheral edge. The micromorphology examination of the isolate demonstrated the presence of hyphae that swell and become multiseptate, budding cells, and lateral conidia were absent. MALD-TOF MS analysis led to the identification of *Trichosporon asteroides* as etiologic. Different treatment regimens were performed with no success, moderate improvement was observed with voriconazole, and treatment is still ongoing. This is the first case report to incriminate *T. asteroides* as an etiological agent of eumycetoma.

Keywords: Mycetoma, *Trichosporon asteroids*, First case

INTRODUCTION

Mycetoma is a neglected disease, endemic, but not restricted to tropical and subtropical areas of the world. As many other neglected diseases, mycetoma affects a poor population living in remote areas; in addition, only a few professionals in the world are well trained to diagnose this infection, there are no adequate diagnostic tools, and only a few therapeutic options are available to treat patients.[1]

Mycetoma is a chronic granulomatous infection of the skin that can progress to deep tissues and structures and eventually spread to other parts of the body, such as muscles and bones.[2-4] Fungus (eumycetoma) or bacteria (actinomycetoma) species are the causative agents of mycetoma. It is recognized that eumycetoma accounts for 40% of cases in the World and actinomycetoma for 60%.[5]

Naturally, these microorganisms live in the soil, in the leaves or thorns of plants; and the infection in humans begins during a traumatic implantation of these microorganisms in the skin, frequently, people working with such materials are the main target of this disease. Therefore, trauma and injuries caused by sharp contaminated materials are believed to be the main route of entry and development of mycetoma.[6]

Around 70 species of microbes are capable of causing mycetoma, but only four are the most common to cause

eumycetoma such as *Leptosphaeria senegalensis*, *Madurella grisea*, *M. mycetomatis* (most common) and *Pseudoallescheria boydii*. In actinomycetoma, the main etiologic species are *Nocardia asteroids*, *N. brasiliensis*, and *N. otidiscaviarum*.[7]

Classically, mycetoma involves the skin and subcutaneous tissue, characterizing the triad of painless subcutaneous swelling, multiple draining sinuses and presence of grains, that are characterized by the presence of bacterial and fungal colonies, which vary in color and size, depending on the infecting organism. The grains consist of microorganisms encapsulated in cement-like material, melanin, and other substances, which may play an important role in protecting microorganisms from the harsh environment induced by the host's inflammatory response, or even protecting the microbe from the drugs used in treatment.[8] In most mycetomas, the size and colour of the grains have some correlation with the infecting species.

Regardless of the infecting species, the clinical manifestation of eumycetoma and actinomycetoma is similar, with subtle differences. The onset and progression of the disease develop more rapidly in actinomycetoma than in eumycetoma, whose lesions increase slowly and exhibit defined margins and remain encapsulated for a long period. In actinomycetoma, lesions are diffuse with no clear margin, are more inflammatory and destructive and can invade deep structures, such as the bones, rapidly after infection.[9] In the late stages of development, mycetoma can cause distortions, deformities, and disabilities in affected members, which are correlated with bone destruction and osteomyelitis and consequently with atrophy and loss of function

In the early phase of infection, the disease appears as a subcutaneous nodule that resembles different infectious or inflammatory conditions, such as botryomycosis, sporotrichosis, or chromoblastomycosis, making an accurate diagnosis extremely difficult.[10-12] However, in chronic lesions, the presence of the clinical triad provides supporting evidence for a specific diagnosis. In this regard, the grains can be useful auxiliary tools for diagnosing the disease, because they can be easily visible discharging from the sinuses and thus can be collected for further mycological analysis. In infections caused by *Madurella* sp., *A. madurae* and *A. pelletieri* large grains can be collected, but they are small in infections with *N. brasiliensis* and *N. asteroids*.[7] In general, black grains have been observed in eumycetoma, while red, yellow, and white grains have been observed in mycetomas caused by bacteria, such as *A. pelletierii*, *Streptomyces somaliensis*, and *A. madurae*, respectively.[13] Fungal hyphae will be observed in potassium hydroxide mount of grains and filamentous bacteria when grains were stained with Gram or acid-fast bacilli.[7]

Imaging exams can also be useful, and can show the extension of lesions, however, some patients show any alteration in the bones. In eumycetoma, lesions tend to form few cavities in bones larger than10 mm, and in actinomycotic cavities they tend to be small, however, they are numerous. Magnetic resonance imaging can be useful; however, it is limited in low-income countries.[14]

In skin fragments processed by the ordinary histological technique and stained with periodic acid Schiff, Gomori methenamine silver, and Gramme stain, it is possible to observe a granuloma with mycetoma grains. Furthermore, different sets of skin fragments can be cultured in the appropriate medium, such as Sabouraud-Dextrose agar with and without antibiotics, blood agar, brain heart infusion agar and Lowenstein Jensen medium.

Biological materials inoculated in the medium are incubated at both 25^0C and 37^0C and colonies growth is observed after 7-10 days, however, delayed growth can be observed, especially if fungi are the etiologic agent, in such cases culture media would be incubated for 4-6 weeks. Depending on the unique morphological characteristics of the culture, the species can be identified.[7]

Molecular diagnosis is a very interesting and effective way to diagnose the etiologic agent, but currently it is limited to commonly used methods, based on 16s RNA gene sequencing for actinomycetes and panfungal PCR for eumycetes. In recent years, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has been used as a tool to identify different species of pathogenic fungi, and compared to other methods, it is reliable and fast.[15,16]

The differentiation of eumycetoma and actinomycetoma is mandatory to guide the best therapeutic option. In this regard, there has been a consensus that there are more available drugs to treat bacterial infection than fungal infection, which in turn is more difficult to treat; and depending on the characteristics of the skin lesions, surgical treatment is indicated to reduce the density of the fungi burden.[17] The usual treatment directed at eumycetoma includes amphotericin B (0.5-3.0 mg/kg/day), itraconazole (400 mg/day), ketoconazole (400 mg/day), posaconazole (800 mg/day), terbinafine (250-500 mg/day), voriconazole (400 mg/day) individually or in combination.[18,19] Azole drugs act on the production of fungal ergosterol by inhibiting the action of 14α-demethylase, leading to accumulation of sterol precursors and resulting in the formation of a plasma membrane with altered structure and function. On the other hand, amphotericin B, a polyene antibiotic, interacts directly with and cleaves the ergosterol molecule, forming a pore, altering the permeability of the fungus.[20] Although allylamine drugs, such as terbinafine, also impact ergosterol production, their mechanism of action differs from that of azoles and polyene antibiotics. The fungal activity of allylamines occurs because they can

inhibit the activity of the squalene epoxidase enzyme, leading to the accumulation of squalene, increasing the permeability of the fungal membrane, leading to the disruption of the cell organization.[21] As is possible to observe, all available drugs act on the route of ergosterol production; therefore, if a given fungal species is a bit more resistant to these classes of drugs, possibly the treatment will last for many months or even years because in fact there are no other fungal drugs capable of binding to other molecules than those belonging to the production of ergosterol.

On the other hand, actinomycetoma is treated with different antibacterial agents, including amikacin, dapsone, trimethoprim-sulfamethoxazole, and streptomycin have been used in different combinations to treat actinomycetoma, depending on the infecting species.[22] Carbapenems, such as imipenem and meropenem, amoxicillin-clavulanic acid, clindamycin, and quinolone, can be used in resistant cases. Unlike the drugs used to treat eumycetoma, all the drugs cited have various mechanisms of action, including inhibition of cell wall, DNA, and protein synthesis.[23] Although there are differences in the treatment of mycetomas caused by fungi and bacteria, current treatment is still limited and suboptimal, given the local and systemic side effects observed during treatment.[24] Furthermore, many patients undergo repeated massive surgical excisions that cause more tissue destruction, fibrosis, and disability.[25-27]

In the present case report, we describe a case of a woman who had undiagnosed skin lesions for 15 years. Taking into account the morphological characteristics of the lesion and microbe, she was diagnosed with eumycetoma. The culture of the fungus allows the morphological identification of *Trichosporon* sp., which, in fact, is new in the literature.

CASE REPORT

The patient is a 56-year-old non-Caucasian woman, born in Itiuba city, Bahia, Brazil, but she has been a resident of the city of São Paulo, Brazil for 30 years. In the last 15 years she observed a progressive increase in the size of the left foot, with the appearance of multiple fistulas on the dorsum of the foot, that emerged a purulent secretion with the presence of small yellowish grains (Figures 1A and 1B). The patient reported that during some visits to her homeland, she suffered small injuries in the left foot, which could be a way to inoculate an etiologic agent into the skin.

In the outpatient dermatology service of the hospital das clínicas da universidade de São Paulo, three deep biopsies were collected in different fistulas. These biopsies were collected once. Biological material was processed using the usual techniques of histology stained with hematoxylin and eosin (HE). The skin scales were incubated with KOH 15% and directly observed in optical microscopy to observed grains; skin fragments were cultured in sabouraud-agar. Furthermore, her serology for HIV and hepatitis A, B, and C was negative. Blood biochemical parameters were also evaluated and were normal.

Colonies of the fungus were subjected to MALDI-TOF MS analysis to identify species of the etiologic agent. A standard protein extraction was performed using ethanol and formic acid and 1µl aliquot of the sample was submitted to MALDI-TOF MS analysis using Vitek MS™ instrument (bioMérieux).[28] For each acquisition group, a standard (Escherichia coli ATCC 8739) was included to calibrate the instrument and validate the run. Spectra were generated using LaunchpadTM v2.8 software (bioMérieux) and analyzed using SARAMIS premium v4.11TM for research use only (RUO) software SARAMIS Premium v4.11™ (bioMérieux).

The histological section stained with H and E exhibited an inflammatory infiltrate composed primarily of mononuclear cells such as macrophages (sometimes reunited in giant cells) and lymphocytes; polymorphonuclear cells were also observed, but in a significantly lower number than mononuclear cells (Figure 1C) fungal grains were surrounded by the inflammatory infiltrate as observed in detail in Figure 1D.

All biological material seeded on Sabouraud agar at 25ºC was positive for *Trichospon* sp. (Figure 2 A), whose colonies exhibited a dry, cream-coloured, cerebriform morphology with a radiated peripheral edge. The fungi grew poorly six weeks after seeding. Micromorphology examination of the isolate demonstrated presence of hyphae that swell and become multiseptate, and the presence of budding cells and lateral conidia was absent. Hyphae and arthroconidia are elongated (Figure 2 B). Furthermore, MALD-TOF MS analysis allowed to correctly identify the species *Trichosporon asteroides* as the etiologic agent with 99.9% confidence level values by comparing the obtained spectra with a new in-house SuperSpectrum library HCFMUSP 02.[29]

Regarding treatment, the patient was initially treated with itraconazole 400 mg/day for four months, after four months, terbinafine (250 mg/day) was associated, but after six months of treatment no improvement was observed in the lesions.

Thus, she was admitted to the dermatology outpatient service of the hospital das clínicas da universidade de São Paulo to receive systemic amphotericin B. She was infused with 3 mg/kg/day during nine days, when she developed severe nephrotoxicity; thus, the treatment was replaced by liposomal amphotericin B. She was infused with 3mg/kg/day of this medication, totalizing an accumulated dose of 962.5 mg, however only a slight improvement was observed after 40 days of the last dose of liposomal amphotericin B, thus the treatment was withdrawn. The combination between intraconazole and terbinafine was restarted in the same dose previously

administered by the physicians; however, after 20 months of treatments no improvement was observed. This treatment was replaced by voriconazole at 400 mg/day for three months. This regimen induced a significant improvement in foot functionality, as well as in the morphological appearance of the lesion; where all fistulas closed and secretion ceased.

During the treatment with imidazole drugs the patient exhibited secondary infections and hepatotoxic episodes, at those moments the medication was suspended and secondary infections were treated with conventional antibiotics, such as cephalexin and ofloxacin. Despite an improvement in the lesions, imidazole treatment is ongoing. The patient is under monthly monitoring and although the skin lesions have healed, the fungi can still be isolated from the skin even after treatment with antibiotic and antimycotic drugs.

The patient was serologically negative for HIV, hepatitis A, B and C, and all serum biochemical parameters were normal.

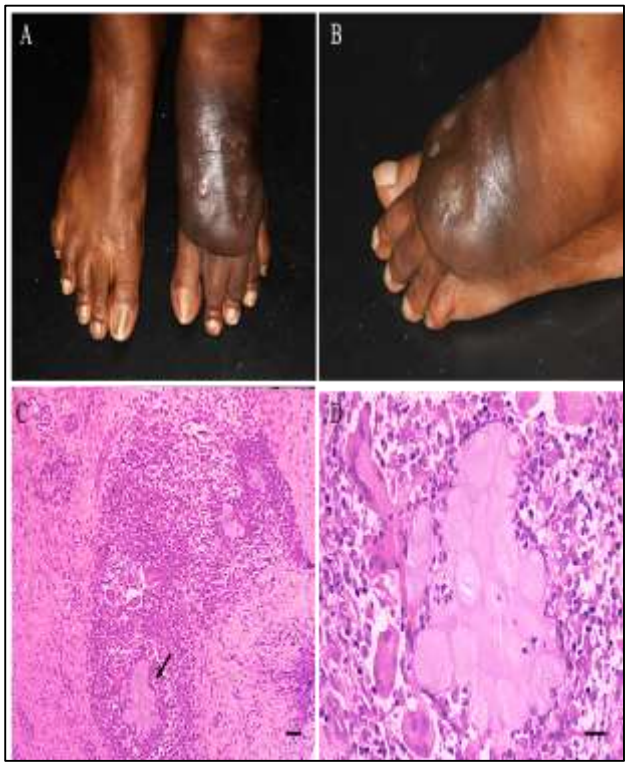

Figure 1 (A-D): A significant increase in volume was observed in the dorsal area of the patient's left foot, exhibiting multiple fistulas, fibrosis, and small yellowish-whitish grains. Histopathologically an inflammatory infiltrate was observed with a predominance of mononuclear cells that surrounded grains in the dermis. The black arrows in C show the presence of grains in the histological sections stained with HE. Magnificence 20x, scale 10xm and magnificence 63x, scale 20xm.

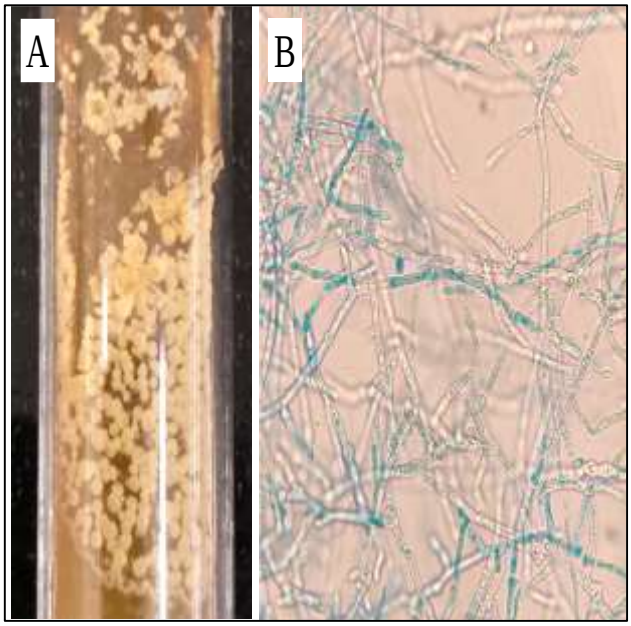

Figure 2 (A and B): Macromorphology of *Trichosporon asteroides* cultivated on Sabouraud agar, incubated at 30°C, exhibiting restrictive colonies, with a dry, cream-coloured and cerebriform aspect. Micromorphology showing that hyphae swell and become multiseptate. Budding cells and lateral conidia are absent. Hyphae and arthroconidia are elongated.

DISCUSSION

Mycetoma is prevalent in poor population in the so-called 'mycetoma belt' that includes Sudan, Somalia, Senegal, Yemen, India, Mexico, and Venezuela, however, other African and Latin American countries have reported cases of these infections such as Egypt, Kenia, Colombia, Argentina and Brazil.[30,31] In general, eumycetoma is prevalent on the African continent, while actinomycetoma is prevalent in Central and South America.[9] Although different in this distribution, the aforementioned patient exhibited a large skin lesion caused by a fungus, characterizing an eumycetoma.

Among the mycetoma cases studied in all affected countries, the most common infecting species is *M. mycetomatis*, which infects around 24.3% of affected patients, followed by *Actinomadura madurae*, *Streptomyces somaliensis*, *Actinomadura pelletieri*, *Nocardia brasiliensis*, and *Nocardia asteroides*.[13] Other less common etiologic agents can also infect humans, such as the bacteria *Streptomyces somaliensis* and *Actinomyces Israeli* as well as the fungi *Pyrenochaeta mackinonii*, *Aspergillus flavus*, *Trichophyton* sp. and *Microsporum* sp.[32,33] However, to our knowledge, there is no record of eumycetoma caused by *Trichosporon asteroides*.

The genus *Trichosporon* can be found in the soil as yeast, or produce superficial or deep infection in humans,

causing white Piedra, onychomycosis, and disseminated infection.[35-37] Specifically, *T. asteroides*, here identified by MALD-TOF MS, has been frequently associated with superficial infection, and so far only a few records have associated this species with disseminated infections, but to our knowledge, this is the first report to associate *T. asteroides* with mycetoma infection.[38] There is a consensus that *T. asteroides* does not form grains; however, this is the first report that incriminates this species as an agent of eumycetoma. Possibly in the deep dermis under pressure of immunological response this fungus species may adapt and form grains. It is still important to note that in histological sections stained with HE, the colony of fungi was surrounded by an intense inflammatory infiltrate composed of mono-, polymorphonuclear, and giant cells. So, in such conditions the parasitic forms of the fungus may adapt to survive in the host by building, for example, a thick layer of cement-like material, as observed in Figure 1 D.

Furthermore, it is important to note that this identification took as basis the morphological characteristics of fungi colonies, i.e., they grew as yeast, with a yellowish-whitish color, typically displaying aspects such as cerebriform and radial surfaces at 25°C. Microscopically, rounded and ovoid arthroconidia with the presence of blastoconidia were observed (Figure 3 and 4), and also by MALD-TOF MS whose spectra matched the internal library built with 93 strains belonging to 16 *Trichosporon* species.[28,29]

CONCLUSION

This is the first case report that incriminates *T. asteroides* as an agent of eumycetoma, in this rare event may explain the difficulty of managing the treatment of this immunocompetent patient.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

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Cite this article as: Belda W, Gimenes VMF, Passero LF. Mycetoma caused by Trichosporon asteroids-report of the first case. Int J Res Med Sci 2023;11:3843-8.