





Chromoblastomycosis

Relationships between laboratorial and clinical data of 12 patients from Rio de Janeiro, Brazil

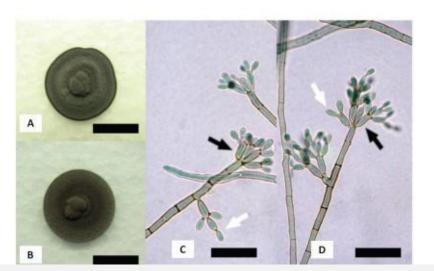
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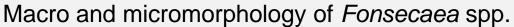




Introduction

- Chromoblastomycosis (CBM) → Neglected Tropical Disease
- Brazil: Genus Fonsecaea
- Fonsecaea spp. are phenotypically very similar, differing only by means of genotypic analyzes



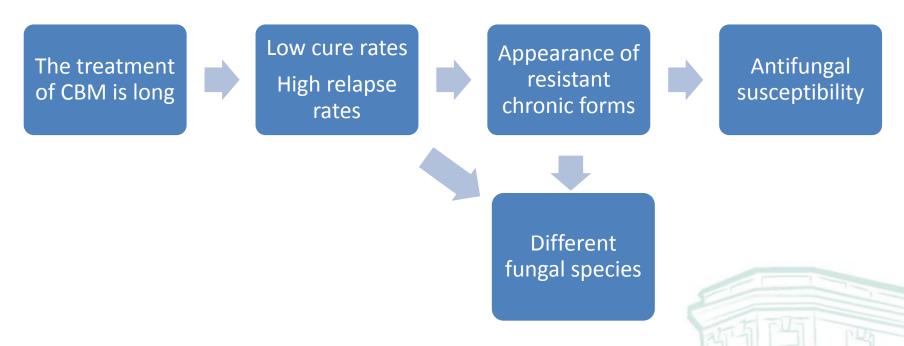








Rationale



• In recent years, new species related to the species traditionally known as CBM agents have been described. For this reason, it is important the genotypic identification of the strains, in order to evidence the prevalence of the species isolated in the study period in our region, as well as to verify if there is variation in the susceptibility profile according to the isolated species.







Patients and Methods

- 12 included patients
- Molecular identification: ITS sequencing
- Antifungal susceptibility: CLSI M38-A2
- Clinical and laboratorial correlation





Patients' Data

- 9 male; 3 female
- Evolution time: 2 months 32 years
- Severity: 8 moderate; 4 severe
- Lesions: 7 verrucous; 5 plaques









Fungal identification

- 8 Fonsecaea monophora
- 3 Fonsecaea nubica
- 1 Fonsecaea pedrosoi

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           NR111612.1 F. multimorphosa CBS980.96
     KP132191.1 F. brasiliensis ISHAM-ITS ID MITS1758
  100 KP132190.1 F. brasiliensis ISHAM-ITS ID MITS1759
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Clinical / Laboratorial correlation

- Severity
 - Moderate CBM
 - 1 F. pedrosoi
 - 5 F. monophora
 - 2 F. nubica
 - Severe CBM
 - 3 F. monophora
 - 1 F. nubica

- Lesions
 - Verrucous
 - 1 F. pedrosoi
 - 4 F. monophora
 - 2 F. nubica
 - Plaques
 - 4 F. monophora
 - 1 F. nubica







Clinical / Laboratorial correlation

- 11 patients: localized lesions
 - 3 using immunosuppressive drugs
 - F. monophora, using tacrolimus
 - F. nubica, using tacrolimus
 - F. monophora, using prednisone
- 1 patient: disseminated lesions
 - F. monophora, using thalidomide





Treatment

- 4 patients: surgical approaches
- 8 patients: itraconazole
 - 5 associated with cryosurgery / surgery / terbinafine / fluconazole









MIC / treatment response correlation

- Fast clinical improvement
 - ITC MIC 0.5 mg/L
 - 3 patients
 - ITC MIC 1.0 mg/L
 - 1 patient

- Slow clinical improvement
 - ITC MIC 0.5 mg/L
 - 1 patient
 - ITC MIC 1.0 mg/L
 - 3 patients







F. monophora infected patient, 58 years, 2 months of CBM evolution









F. monophora infected patient, 72 years, 32 years of CBM evolution







Remarkable findings

- High rate of F. monophora infected patients
 - All raised and born in RJ are infected with this species
- Absence of cerebral involvement in F. monophora infected patients
- Fast diagnosis leading to mild cases, even in immunosuppressed patients







Implication in Laboratorial Diagnosis

• Isolation of the CBM agents in culture from clinical specimens is strongly recommended to allow clinical and laboratorial correlation studies in this mycosis.



PDA plate after 7 days of incubation at 30 °C.



Micromorphological aspect of F. pedrosoi strain observed by the slide culture.









Molecular identification and antifungal susceptibility profiles of clinical strains of *Fonsecaea* spp. isolated from patients with chromoblastomycosis in Rio de Janeiro, Brazil

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Data Availability Statement: The authors confirm that all data underlying the findings are fully available without restriction. The sequences obtained in this study have been deposited in GenBank under the accession numbers MF616485 – MF616504. All other relevant data are within the paper and its Supporting Information files.

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Abstract

Background

Chromoblastomycosis (CBM) is a difficult-to-treat chronic subcutaneous mycosis. In Brazil, the main agent of this disease is Fonsecaea pedrosoi, which is phenotypically very similar to other Fonsecaea species, differing only genetically. The correct species identification is relevant since different species may differ in their epidemiologic aspects, clinical presentation, and treatment response.

Methodology/Principal findings

Partial sequencing of the internal transcribed spacer (ITS) was used to identify twenty clinical isolates of Fonsecaea spp. Their in vitro antifungal susceptibility was determined using the broth microdilution method, according to the M38-A2 protocol. Amphotericin B (AMB), flucytosine (5FC), terbinafine (TRB), fluconazole (FLC), itraconazole (ITC), ketoconazole (KTC), posaconazole (POS), voriconazole (VRC), ravuconazole (RVC), caspofungin (CAS), and micafungin (MFG) were tested. The association between ITC/TRB, AMB/5FC, and ITC/CAS was studied by the checkerboard method to check synergism. The available patients' data were correlated with the obtained laboratory results. Fonsecaea monophora (n = 10), F. pedrosoi (n = 5), and F. nubica (n = 5) were identified as CBM' agents in the study. TRB and VRC were the drugs with the best in vitro activity with minimal inhibitory concentrations (MIC) lower than 0.25 mg/L. On the other hand, FLC, 5FC, AMB, and MFG showed high MICs. The AMB/5FC combination was synergistic for three F. monophora strains while the others were indifferent. Patients had moderate or severe CBM, and ITC











MOLECULAR IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY PROFILES OF CLINICAL STRAINS OF Fonsecaea spp ISOLATED IN RIO DE JANEIRO, BRAZIL

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INTRODUCTION

Chromobiastomycosis (CBM) is a chronic mycosis that affects the skin and the subcutaneous tissues, infection usually follows a human trauma with a contaminated organic material such as plant thoms, wood, plant debris, among others, leading to the implantation of the furgus in the subcutaneous tissues, where the fungus changes to its parasitic form composed by muriform cells [1]. in Brazil, species of the genus Fonsecaea are the main agents of CBM, which are phenotypically very similar, differing only by means of genotypic analyzes. This disease can be caused by four Fonserage species: F. pediosol, F. nubica [2-4], E monophore and E pugnacius. The later two show significant neurotropism, elentually leading to dissemination to the brain and other organs [2,5] or causing primary brain infection without skin lesions, which are classified as phaeohyphomycosis since no muriform cells are seen in tissues [1,5,6]. CBM may assume several clinical forms with different degrees of severity [1]. There is no treatment protocol to be followed, and antifungal therapy is often combined with physical methods such as cryosurgery or surgical excision for small lesions (7). Itraconazole (ITZ) and terbinafine (TRB) are the most used drugs in the treatment of CBM [1,8-10]. In addition, combined therapies of ITZ with TRB [1], SFC with AMB [11], or ITZ with SFC have been used [12]. It is important to determine the in vitro susceptibility of these isolates because of the difficulty found in the treatment of this mycosis and the frequency of refractory cases and relapses. The objectives of this study were to identify genotypically and to evaluate the In vitro susceptibility to antifungals of 20 Fonsecaea spp. Strains obtained from 17 patients with CBM in Rio de Janeiro, Brazil.

MATERIAL AND METHOD 8



Molecular characterization was performed through the amplification and sequencing of the ITS1-5.88-ITS2 region of IDNA [14-17].



In vitro susceptibility: The minimum inhibitory concentration (MIC) of amphotencin B (AMB), flucytosine (SFC), terbinatine (TRB), fluconazole (FLZ), traconazole (ITZ), ketoconazole (KTZ), posaconazole (PSZ), voriconazole (VRZ), and ravuconazole (RVZ) or the minimum effective concentration (MEC) of caspofungin (CAS) and micafungin (MFG) were determined by the broth microdilution method, according to the Clinical and Laboratory Standards institute M38-A2 protocol, with modifications [18].



RESULTS

Three Fonsecaea species were identified: F. monophora (n = 10), F. pedrosol (n = 5), and F nublca (n = 5). Regarding the antifungal susceptibility test, TRB and VRZ were the antifungal drugs that yield the lowest MICs (geometric means: 0.09 and 0.14 µg/ml, respectively). On the other hand, FLZ, SFC, AMB, and MFG showed high MICs/MEC (geometric means: 12.55, 6.28, 5.77, and 5.66 µg/ml, respectively). All patients presented mild, moderated or severe CBM, and none had cerebral involvement, including those infected with F. monophora. Antifungal therapy with ITZ was ineffective in most cases, regardless of strain MIC to this antifungal drug. These cases required cirurgical approaches during patient's management.



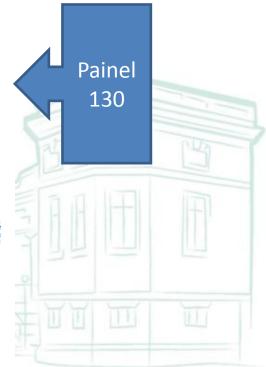
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CONCLUSIONS

These results point to a predominance of F. mongohora in Rio de Janeiro, which is the second Fonsecaea species of South America. The absence of cerebral involvement in all patients infected with R monophore suggests that neurotropism of this species must be strain dependent. TRB and VRZ should be better studied in the clinical context of CBM treatment due to their low MICs.

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