Chromoblastomycosis

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

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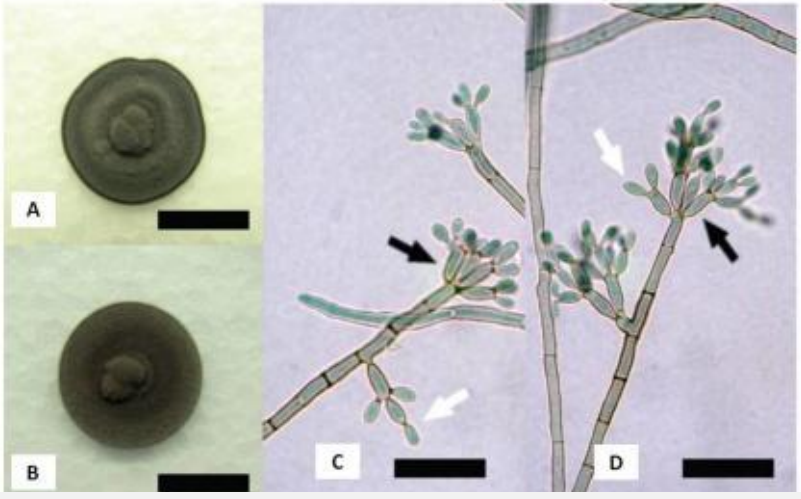
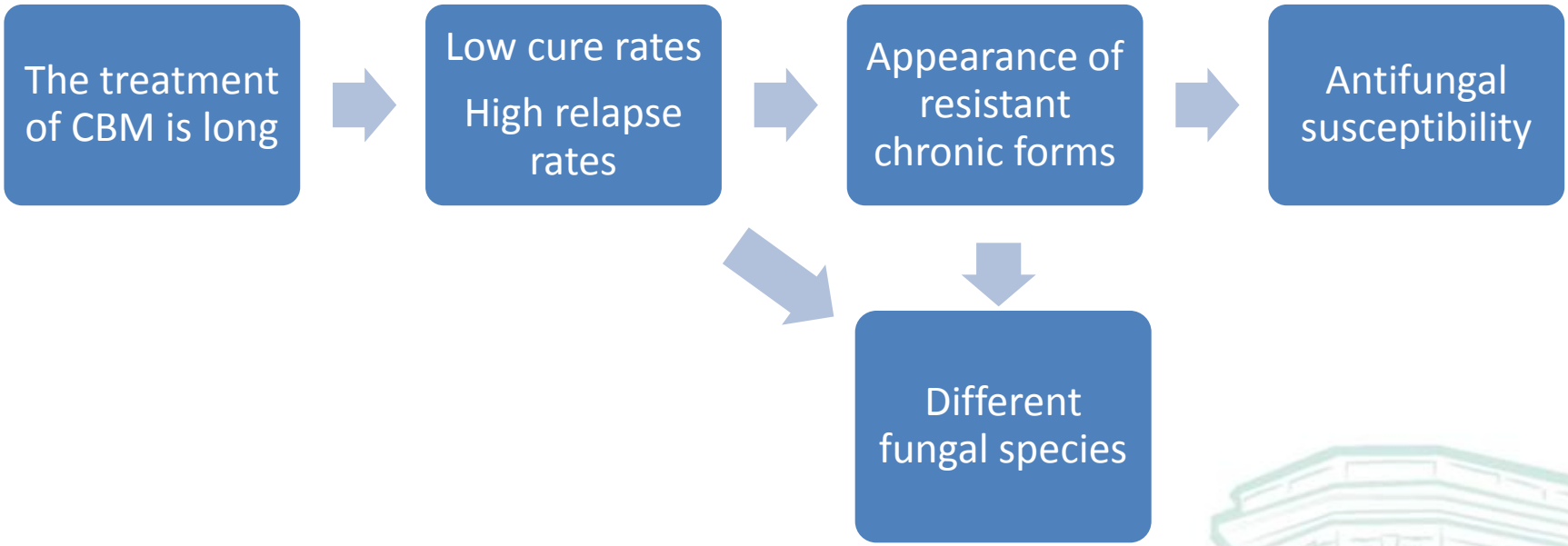
Introduction

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



Macro and micromorphology of *Fonsecaea* spp.

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

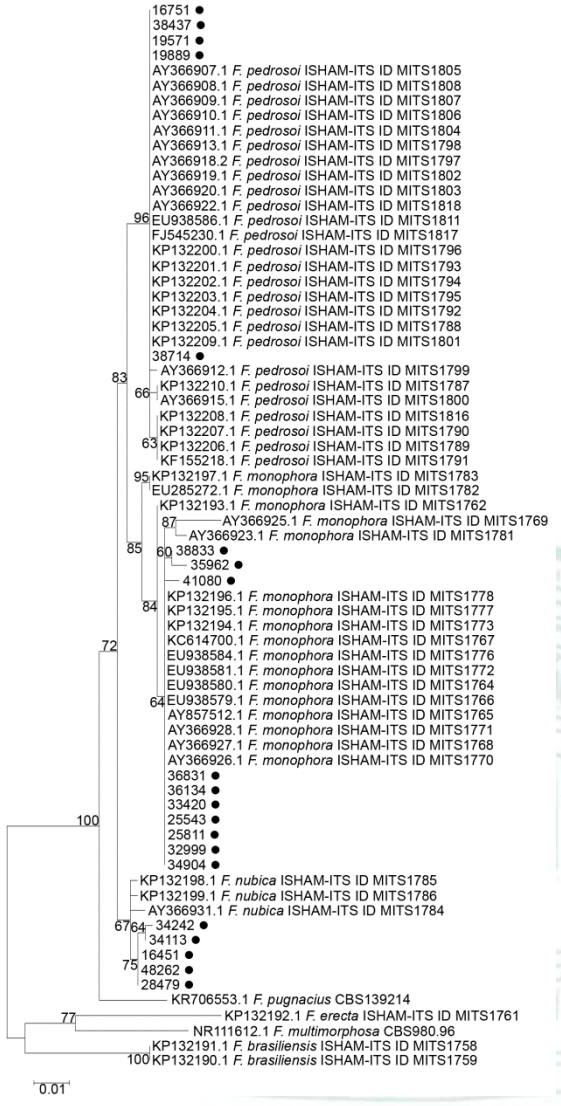
- 12 included patients
- Molecular identification: ITS sequencing
- Antifungal susceptibility: CLSI M38-A2
- Clinical and laboratorial correlation

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*



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

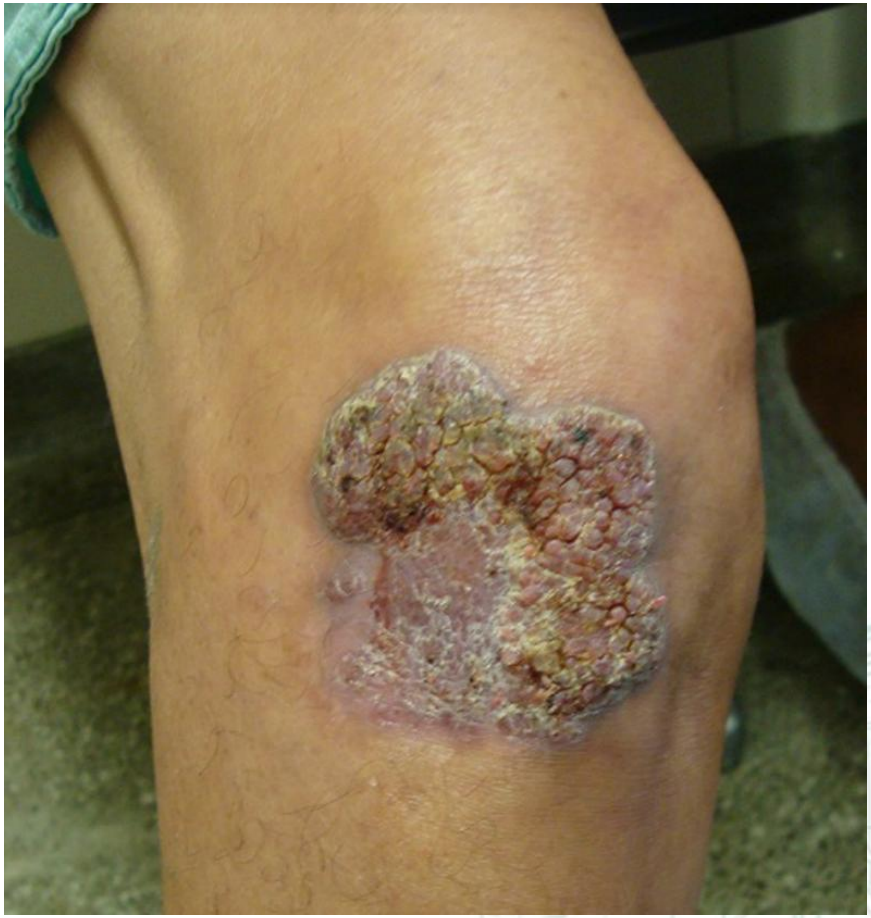
Treatment

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

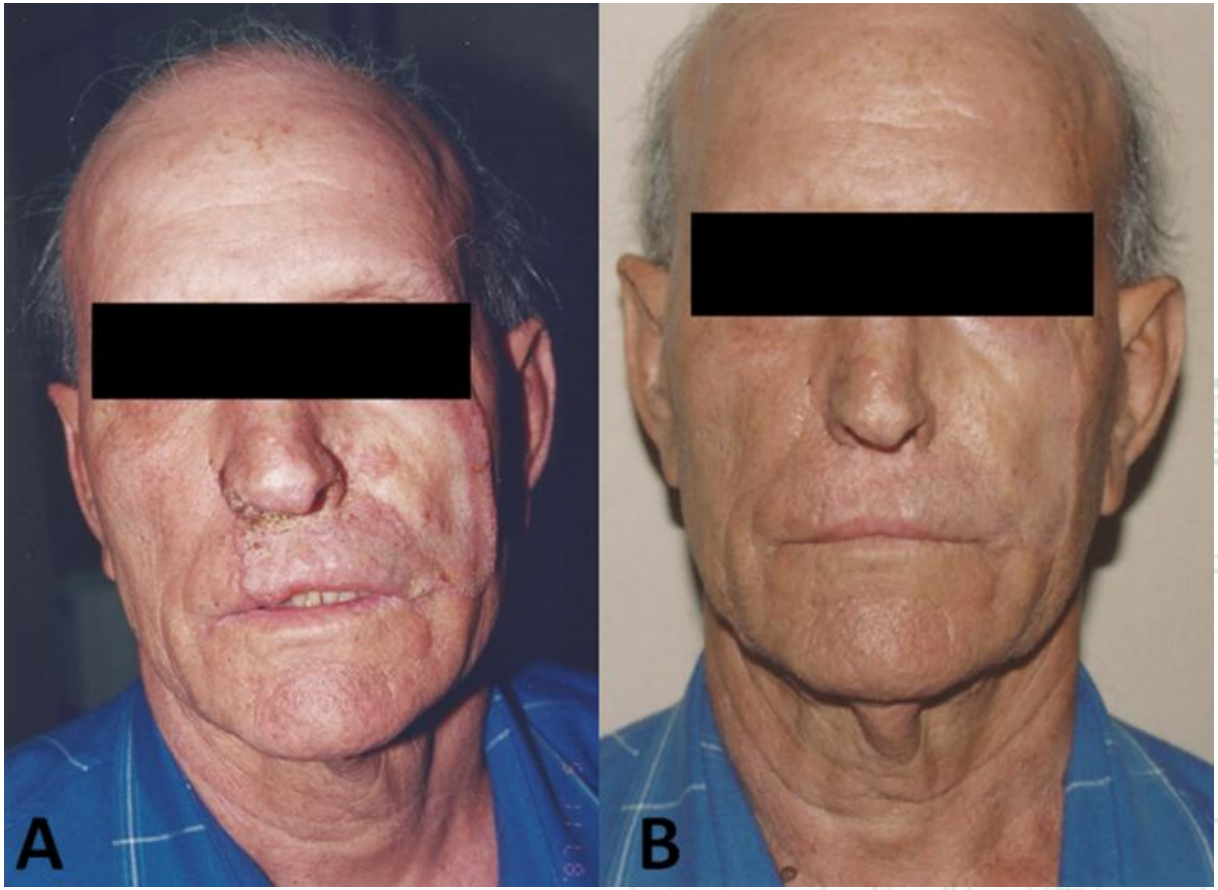
MIC / treatment response correlation

- 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.

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

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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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

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

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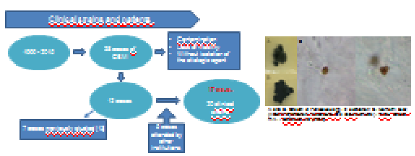
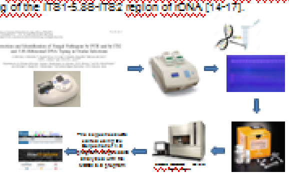
INTRODUCTION

Chromoblastomycosis (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 thorns, wood, plant debris, among others, leading to the implantation of the fungus 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 *Fonsecaea* species: *F. pedrosoi*, *F. nubica* [2–4], *F. monophora* and *F. pugnacius*. The latter two show significant neurotropism, eventually 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], 5FC with AMB [11], or ITZ with 5FC 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.

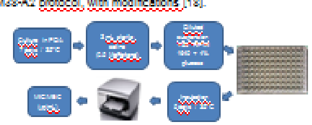
MATERIAL AND METHODS



Molecular characterization was performed through the amplification and sequencing of the ITS1-5.8S-ITS2 region of rDNA [14-17].

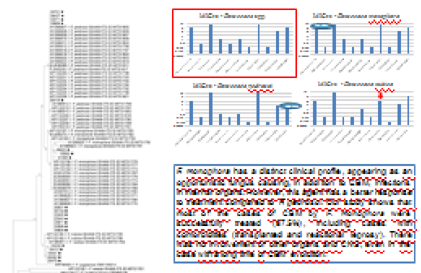


***In vitro* susceptibility**: The minimum inhibitory concentration (MIC) of amphotericin B (AMB), flucytosine (5FC), terbinafine (TRB), fluconazole (FLZ), itraconazole (ITZ), ketoconazole (KTZ), posaconazole (PSZ), voriconazole (VRZ), and ravuconazole (RVZ) or the minimum effective concentration (MEC) 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. pedrosoi* (n = 5), and *F. nubica* (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, 5FC, 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.



CONCLUSIONS

These results point to a predominance of *F. monophora* in Rio de Janeiro, which is the second *Fonsecaea* species of South America. The absence of cerebral involvement in all patients infected with *F. monophora* 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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Painel 130

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