

TITLE: GENOTYPING OF *Paracoccidioides brasiliensis* FROM PATIENTS SAMPLES TREATED IN BOTUCATU MEDICAL SCHOOL'S HOSPITAL FROM 2004 TO 2014.

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ABSTRACT

Paracoccidioidomycosis is a chronic granulomatous mycosis most prevalent in Latin America, that until recently was believed to be caused only by *Paracoccidioides brasiliensis* (*P. brasiliensis*). However, in 2006, researchers described three cryptic species: S1, PS2, and PS3, and in 2009, *Paracoccidioides lutzii* (Pb01-like). All these agents are thermomorphogenic fungi that develop as yeast *in vivo*, in host tissues or *in vitro* cultures at 37°C in culture media. It also grows as mycelium at room temperature ranging from 4 to 28°C. These species are not uniformly distributed in Latin America, some are more prominent in some regions than in others. Due to the existence of sympatric cryptic species of *P. brasiliensis*, further analyses of patient samples are needed for the better understanding of the distribution and occurrence of the recently species in Botucatu city region. This will favor a possible correlation between genetic groups and mycological and clinical characteristics. Therefore, the genotyping of clinical specimens from patients that have been diagnosed with paracoccidioidomycosis treated in Botucatu Medical School's Hospital, may contribute to epidemiological and clinical studies of this disease. It was performed techniques for the removal of the paraffin in the slices of the biopsies, followed by extraction of DNA, PCR and sequencing of four target genes (ITS, CHS2, GP43 and ARF). At first, we gathered 184 samples from 2004 to 2014, obtaining only 44 positive and sequenced. The samples were blasted at <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. Our data collected from the sequences of ARF and CHS2 genes showed that 100% of the 44 positive samples are from S1 cryptic species which corroborate with literature. The GP43 gene data and the comparison with the clinical information are being analyzed. We conclude our samples are a challenge to successfully extract good quality DNA.

Keywords: Cryptic species, *Paracoccidioides brasiliensis*, sequencing.