

MOLECULAR IDENTIFICATION OF PARACOCCIDIOIDES SPECIES ISOLATED FROM PATIENTS AND THE ENVIRONMENT OF THE STATES OF SÃO PAULO, MINAS GERAIS AND PARANÁ, BRAZIL.

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Recently, four (4) new species of etiological agents that cause paracoccidioidomycosis (PCM) have been proposed. These species are *Paracoccidioides brasiliensis sensu stricto* (S1a and S1b), *P. americana* (PS2), *P. restrepensis* (PS3), *P. venezuelensis* (PS4), species previously belonging to the *P. brasiliensis* complex, besides *P. lutzii*. This new characterization is based on phylogenetic, geographic and micromorphological differences between the etiological agents of PCM. The species *P. brasiliensis sensu stricto* is well elucidated in the literature, and is one of the causes of the infection that can evolve into the two forms of PCM (chronic and acute-subacute). In this study, clinical isolates of patients from the Hospital das Clínicas of Ribeirão Preto – HCFMRP/USP and environmental isolates were genotyped to better understand the geographical distribution and their biological characteristics. To confirm the genus *Paracoccidioides* spp, the genomic mycelial DNA from the clinical and environmental isolates was subjected to PCR (Polymerase Chain Reaction) for the amplification of the GP43, 43-kD gene immunodominant glycoprotein that codes for an antigen used for PCM diagnosis. After the confirmation of the genus, the identifications of the species *P. brasiliensis sensu stricto*, *P. americana*, *P. restrepensis* and *P. lutzii* were performed by PCR - RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) method with the amplification of the α - Tubulin coding gene with the double digestion with the restriction endonucleases, *BclI* and *MspI*. Twenty isolates (19 clinical and 1 environmental), all identified as belonging to the species *P. brasiliensis sensu stricto*, where the *BclI* enzyme recognized its restriction site yielding two (2) fragments, one (1) of 155 bp and one (1) of 108 bp. In Brazil, the geographical distribution of *P. brasiliensis sensu stricto* comprises the southern and southeastern regions of the country. The result confirmed the prevalence of this etiological agent in cities in the northeastern region of the State of São Paulo, southern Paraná State and southern Minas Gerais State, in the southern and southeastern regions of Brazil. The clinical form of the PCM of the patients from which the isolates of *P. brasilienseis sensu stricto* genotypes were obtained were classified as chronic (66.66%) and acute-subacute (33.34%). Genotyping using the PCR-RFLP technique of the α -Tubulin gene is a powerful tool that can quickly and effectively help identify the etiological agents of PCM when compared to standardized tests used in large medical centers in Brazil. We can correlate the genotypic data with the clinical, evolutionary and epidemiological aspects obtained from medical records. It is expected that these results will bring a contribution relative to the epidemiology and pathogenesis of PCM.

Keywords: Paracoccidioides spp., Genotyping, PCR-RFLP

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