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Paracoccidioides brasiliensis PS2: First Autochthonous Paracoccidioidomycosis Case Report in Rio de Janeiro, Brazil, and Literature Review

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Abstract Paracoccidioidomycosis (PCM) is a systemic mycosis caused by pathogenic dimorphic fungi of the *Paracoccidioides brasiliensis* complex. It is the most important systemic mycosis in Latin America, mainly in Brazil. Despite its severity and high mortality rates, it is considered a neglected disease. Species within the genus *Paracoccidioides* present genetics and morphological variations with probable clinical, diagnostic and therapeutic consequences. In fact, there are a very small number of detailed case reports with molecular identification of these fungal agents. Here, it is reported a case of PCM due to *Paracoccidioides brasiliensis* PS2. Molecular identification of the isolate was performed by amplification and sequencing of the *arf* and *gp43* genes. Clinical cases and strain reports with molecular identification in the literature are also reviewed. The case herein presented is the first autochthonous report of PCM due to *Paracoccidioides brasiliensis* PS2 species in the state of Rio de Janeiro, Brazil, an important endemic area. The patient presented a chronic pulmonary form of PCM and had a satisfactory response to sulfamethoxazole/trimethoprim although sequelae such as adrenal insufficiency and dysphonia were observed. This study may contribute to improve the knowledge about this severe disease, its causative cryptic species and their consequences to patients.

Keywords Paracoccidioidomycosis · *Paracoccidioides brasiliensis* PS2 · Molecular identification · Pulmonary chronic disease

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Introduction

Paracoccidioidomycosis (PCM) is the most important systemic mycosis in Latin America, especially in Brazil, from where the majority of the cases are reported [1]. In this country, PCM is the eighth cause of death among chronic or recurrent infectious and parasitic diseases [2] and the most prevalent systemic mycosis, covering 35 % of the Brazilian territory [3].

Two major clinical manifestations have been observed in PCM disease: an acute or juvenile type that affects both gender of any age; and a chronic or adult type with lung, skin and mucosal involvement in

male adults predominantly [1]. Despite its high prevalence and mortality, PCM is not classified as a mandatory reportable disease. Moreover, the paucity of access to diagnosis and treatment characterizes PCM as a serious neglected disease in Brazil [3].

For almost a century, PCM was thought to be caused by the single species *Paracoccidioides brasiliensis* [1]. However, a phylogenetic study indicated that this species consists in fact of three distinct species named *P. brasiliensis* S1, PS2 and PS3 [4]. Further phylogenetic studies based on the genealogical concordance method of phylogenetic species recognition (GCPSR) revealed that the best studied *P. brasiliensis* strain in Brazil, Pb01, and other 16 strains comprise a clade totally distinct from the previously described S1/PS2/PS3 clade [5]. This so-called Pb01-like group is now recognized as the new species *Paracoccidioides lutzii* [6]. Moreover, a new cryptic phylogenetic species, PS4, was also recently described [7, 8].

The species *P. brasiliensis* S1 and PS2 are very closely related phylogenetically [9, 10]. They share morphological characteristics of both yeast and mycelial phases [11, 12], genomic structure [13], low expression of genes related to sexual reproduction [14], in vitro susceptibilities to the combination sulfamethoxazole/trimethoprim (SMZ/TMP) and to other antifungal drugs [15–20], and also families of transposable elements in the genome [21]. On the other hand, significant differences in metabolism pathways [22], antigenic composition [23] and gp43 expression (the main exocellular antigen recognized by sera from patients with paracoccidioidomycosis) [24] are observed among them as well.

It has been described that *P. brasiliensis* and *P. lutzii* have differences in terms of conidial and yeast morphology [6, 25, 26], and also antigenic composition [27]. In fact, different serological responses are noticed in patients with PCM caused by *P. brasiliensis* or *P. lutzii* when antigens from these two species are used in serological tests [8, 28]. It is also speculated that disease caused by the cryptic species of the genus *Paracoccidioides* may present differences in severity, tissue involvement and response to therapy [8, 29]. In this paper, we report the first autochthonous case of chronic pulmonary PCM caused by *P. brasiliensis* PS2 in a patient from Rio de Janeiro, Brazil, an important endemic area of this mycosis [30].

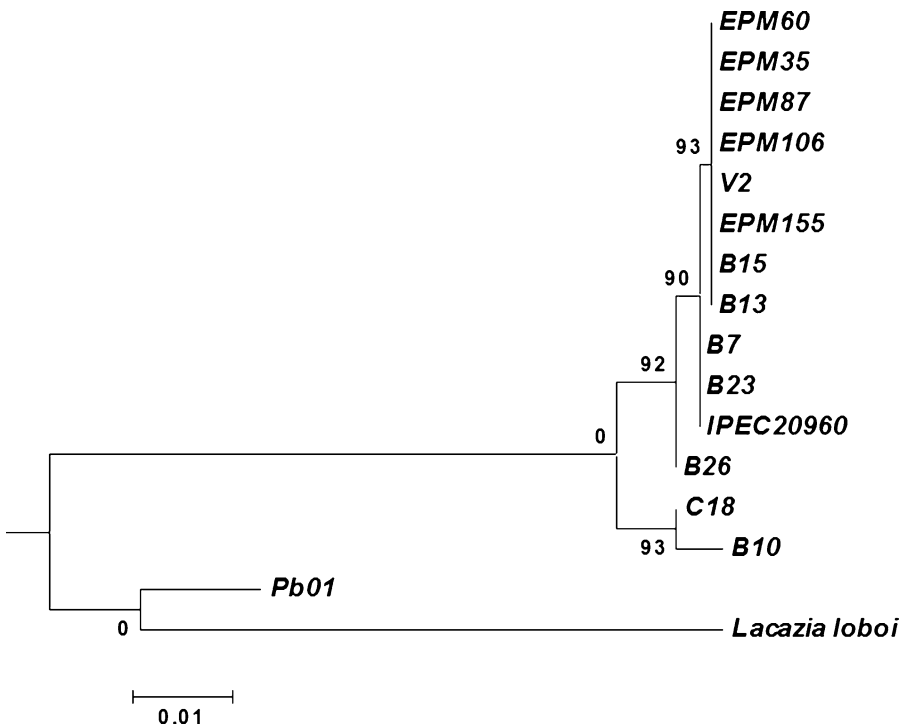

Fig. 1 Phylogenetic relationships of *arf* and *gp43* genes among the clinical isolate of this case report and other 11 *P. brasiliensis* PS2 strains deposited at GenBank. One *P. brasiliensis* S1 (B10 strain), one *P. brasiliensis* PS3 (C18 strain), one *P. lutzii* (Pb01 strain) and *Lacazia loboi* were included in the analysis. The phylogenetic tree was estimated in the SeaView version 4 software. Bootstrap values after 1000 replicates are presented in the branch nodes

Table 1 PCM cases reported in the literature with molecular characterization of the causative agent and clinical aspects of the patient

Reference	Number of cases	Clinical form	Identified species
Hahn et al. [32]	1	Acute PCM	*P. lutzii*
Marques-da-Silva et al. [33]	2	Chronic PCM	*P. lutzii*
Ballesteros et al. [34]	1	Acute PCM	*P. brasiliensis*
Macedo et al. [35]	1	Acute PCM	*P. brasiliensis* S1

Case Report

A 42-year-old man presented to Evandro Chagas National Institute of Infectious Diseases complaining of hoarseness, non-quantified weight loss, mucosal lesions and mild dyspnea in the preceding 6 months. The patient was referred from Casimiro de Abreu, Rio de Janeiro State, Brazil (22°28′51″S and 42°12′14″W). He reported to be a former farmer in this city and was denied recent or previous travels to other states in the country. Important medical history information to notice was as follows: seizures due to epilepsy since childhood controlled with phenobarbital and carbamazepine, tobacco smoking (8 pack-years), alcohol use disorder and chronic hepatitis B. Reticular interstitial pattern was present in chest radiology, cranial computerized tomography did not present suggestive images of fungal infection, and laboratory analyses (hematology and biochemistry) were within normal ranges, except the ACTH (cosyntropin) stimulation test: 14.48 μg/dL (reference value higher than 18–20 μg/dL after 30 min). The results of Anti-HIV ELISA serological test was negative. Pulmonary tuberculosis was ruled out by negative Ziehl–Neelsen staining and Lowenstein-Jensen culture of the sputum. The PCM diagnosis was confirmed by a positive direct KOH examination of the sputum; isolation and identification of the fungus from samples of mucosal lesions; and also positive antibodies detection for PCM in Ouchterlony immunodiffusion test (ID) (1:8). Genomic DNA was obtained from the yeast phase according to Ferrer et al. [31]. Automated partial nucleotide sequencing of two protein-encoding genes was done in the sequencing platform at Fundação Oswaldo Cruz—PDTIS/FIOCRUZ, based on amplicon products of polymerase chain reaction (PCR) using the *arf* (ADP-rybosilation factor) and *gp43* (glucan 1,3-beta-glucosidase) primers [4, 6]. The sequences of the isolate (IPEC 20960) were deposited in GenBank (KU645890 and KU645891 for *gp43* and *arf* loci, respectively), and a BLAST analysis (www.ncbi.nlm.nih.gov/BLAST) comparing these sequences with those from isolates belonging to the *Paracoccidioides brasiliensis* complex previously deposited by Matute et al. [4] presented 100 % similarity with *P. brasiliensis* PS2 strain T10B1 (B7) (Fig. 1). The patient presented clinical, imaging and serological cure after receiving sulfamethoxazole/trimethoprim (SMZ/TMP 800/160 mg b.i.d) for 3 years. He was followed up for 4 years after drug suspension due to dysphonia and low adrenal reserve sequelae that lead to the need of continuous phonoaudiology and endocrinology support.

Discussion

Despite the clinical relevance that may be hidden within the cryptic species of the *Paracoccidioides* genus, there is a paucity of PCM case reports in the literature with molecular characterization of the strains (Table 1). Two of these reports describe *P. lutzii* as the agent of two severe PCM cases, one of which with a fatal outcome, and a third mild case, in which the patient presented emaciation, mucosal lesions and ganglia, but without fever or cough [32, 33]. Another report describes an acute *P. brasiliensis* case in Colombia, where the fungus was identified by molecular methods, but without description of its phylogenetic species [34]. More recently, Macedo et al. [35] described an acute and severe paracoccidioidomycosis case due to *P. brasiliensis* S1 that was misdiagnosed as hypereosinophilic syndrome because of a high eosinophil count in peripheral blood and a massive splenomegaly. To the best of our knowledge, there are no case reports of PCM due to the *P. brasiliensis*

Table 2 Strains of *P. brasiliensis* PS2 described in the literature

Strain		Identification method	Studies	References
Original ID	Other ID			
1956Uruguai	EPM35	MLST, PCR-RFLP	Molecular identification	Roberto et al. [36]
1925	EPM60	MLST, PCR-RFLP	Molecular identification	Roberto et al. [36]
Pb320	EPM87	MLST, PCR-RFLP	Molecular identification	Roberto et al. [36]
LDR4	EPM106	MLST, PCR-RFLP	Molecular identification	Roberto et al. [36]
Pb77	EPM141	PCR-RFLP	Molecular identification	Roberto et al. [36]
DAS	EPM155	MLST, PCR-RFLP	Molecular identification	Roberto et al. [36]
Pb1087	EPM168	PCR-RFLP	Molecular identification	Roberto et al. [36]
Pbdog	EPM194	PCR-RFLP, SNaPshot, qPCR	Molecular identification	Roberto et al. [36]
			Speciation and biogeography	Theodoro et al. [11]
			Dimorphism and virulence	Theodoro et al. [40]
Pb03	B26, EPM210	MLST, PCR-RFLP	Molecular identification	Roberto et al. [36]
			Genomics	Muñoz et al. [10], Desjardins et al. [13]
			Antifungal susceptibility Sexual reproduction Speciation and biogeography Yeast-cell morphology	Cruz et al. [15], Johann [17], Lima et al. [18], Johann [19], Campos et al. [20]
			Transposable elements gp43 polymorphisms	Teixeira et al. [38], Gomes-Rezende et al. [14]
			Molecular typing	Theodoro et al. [11]
			Phylogeny	Menino et al. [12]
				Marini et al. [21]
				Rocha et al. [24]
				Matute et al. [9]
				Matute et al. [4]
LDR3	IFM54649	Unknown	Serologic study	Lenhard-Vidal et al. [23]
Pb02	V2	MLST	Antifungal susceptibility Proteomics Sexual reproduction Speciation and biogeography Yeast-cell morphology	Cruz et al. [15], de Paula e Silva et al. [16], Johann et al. [17], Lima et al. [18], Campos et al. [20]
				Pigosso et al. [22]
				Teixeira et al. [38]
			Protein expression	Theodoro et al. [11]
			Transposable elements	Menino et al. [12]
			Molecular typing	García Blanco et al. [45]
			Phylogeny	Marini et al. [21]
				Matute et al. [9]
				Matute et al. [4]
T10B1	B7	MLST, SNaPshot, qPCR	Sexual reproduction	Teixeira et al. [38]
			Speciation and biogeography	Theodoro et al. [11]
			Protein expression	García Blanco et al. [45]
			Dimorphism and virulence	Theodoro et al. [40]
			Molecular typing	Matute et al. [9]
			Phylogeny	Matute et al. [4]

Table 2 continued

Strain		Identification method	Studies	References
Original ID	Other ID			
Pb927	U1	SNaPshot, qPCR	Speciation and biogeography	Theodoro et al. [11]
			Phylogeny	Matute et al. [4]
Bt84	B15	MLST, SNaPshot, qPCR	Sexual reproduction	Teixeira et al. [38]
			Speciation and biogeography	Theodoro et al. [11]
			Protein expression	García Blanco et al. [45]
			Dimorphism and virulence	Theodoro et al. [40]
			Molecular typing	Matute et al. [9]
			Phylogeny	Matute et al. [4]
Pb262		MLST, SNaPshot, qPCR	Speciation and biogeography	Theodoro et al. [11]
Pb04	B23	MLST, qPCR	Sexual reproduction	Teixeira et al. [38]
			Antifungal susceptibility	Cruz et al. [15], Johann et al. [17], Lima et al. [18], Campos et al. [20]
			Speciation and biogeography	Theodoro et al. [11]
			Yeast cell morphology	Menino et al. [12]
			Transposable elements	Marini et al. [21]
			Molecular typing	Matute et al. [9]
			Phylogeny	Matute et al. [4]
1430		SNaPshot	Speciation and biogeography	Theodoro et al. [11]
Pb106		SNaPshot, qPCR	Speciation and biogeography	Theodoro et al. [11]
Pb22		SNaPshot, qPCR	Speciation and biogeography	Theodoro et al. [11]
Uberlândia	B13	MLST	Protein expression	García Blanco et al. [45]
			Molecular typing	Matute et al. [9]
			Phylogeny	Matute et al. [4]
IPEC20960		MLST	Case report	This study

phylogenetic species PS2, PS3 or PS4 describing clinical and therapeutic aspects of the patients.

The case herein described was caused by *P. brasiliensis* PS2. The number of *P. brasiliensis* PS2 strains in the literature is small, when comparing with the phylogenetic species S1 and PS3 [9, 36]. It was found, in a literature review, 13 papers describing 20 different strains (Table 2) from four different countries (Brazil, Argentina, Venezuela and Uruguay) belonging to this phylogenetic species (Fig. 2). Besides, two PCM cases were assigned to *P. brasiliensis* PS2 by the sequencing of the product of a semi-nested PCR targeting the gp43 gene, but without isolation of the fungus in culture [37]. These works showed that *P. brasiliensis* PS2 is a paraphyletic [9], heterothallic species [38] that can be differentiated from S1 by multilocus sequence typing (MLST) [4, 39], microsatellite evaluation [9], restriction fragment length polymorphism of the α-tubulin gene using the *Msp*I and *Bcl*I restriction enzymes [36], and single nucleotide polymorphism analysis that can be performed by quantitative real-time polymerase chain reaction (qPCR) or SNaPshot® [11]. In the present work, the strain was successfully identified as *P. brasiliensis* PS2 by the sequencing of *gp43* and *arf* genes according to methodologies described by Matute et al. [4] and Teixeira et al. [6].

Initially, virulence of *P. brasiliensis* PS2 was thought to be lower than *P. brasiliensis* S1 [9]. The supposed lower virulence of *P. brasiliensis* PS2 might lead to mild PCM cases. However, other studies demonstrated that *P. brasiliensis* PS2 strains can

Fig. 2 Geographic distribution of *P. brasiliensis* PS2 strains described in the literature. Cities marked in yellow or in red present one or two strains, respectively. The orange marker represents Casimiro de Abreu municipality, place of birth and residence of the patient described in this study

present high and intermediary virulence, similarly to *P. brasiliensis* S1 [40], which may yield PCM presenting more severe symptoms and sequelae. The case reported herein reinforces this finding, since the patient infected by *P. brasiliensis* PS2 had adrenal insufficiency, a serious complication of PCM [41]. Moreover, the patient presented dysphonia, another frequent sequel of this mycosis [42].

It is also important to consider that a good clinical therapeutic response to SMZ/TMP was previously supposed to be more related to *P. lutzii*. However, the case herein discussed also presented a satisfactory outcome after receiving these drugs.

The positive antibody detection in the ID test performed in this case with mixed exoantigens of strains Pb01 and Pb18, *P. lutzii* and *P. brasiliensis* S1, respectively [8], shows that these two species can be used for *Paracoccidioides* antigen preparations for serologic tests to detect antibodies in infections caused by *P. brasiliensis* PS2. Small quantities of the antigen gp43 were found in culture filtrates of *P. lutzii* strains, and this molecule appeared to be more variable within *P. lutzii,* suggesting a different evolutionary process. However, the variation on gp43 production also occurs between isolates belonging to the same species, indicating that speciation events are important, but not enough to explain the diversity in terms of antigen production between the cryptic species and nonreactive results by immunodiffusion assays [43]. Therefore, it is recommended that different antigenic preparations from several *P. brasiliensis* complex species should be applied to improve PCM serodiagnosis by decreasing the number of false-negative patients seen in other studies [8, 44]. One candidate molecule to be used as antigen in the serodiagnosis of PCM is the *Pb*P27 protein produced by *P. brasiliensis* S1, PS2, PS3, and *P. lutzii* especially in the parasitic yeast phase [45].

Although there is a paucity of case reports due to this phylogenetic species, seven *P. brasiliensis* PS2 strains (35 %) are described in the literature as isolated from patients with the chronic form of PCM [9, 11, 15, 36], as observed in our case. One PS2 strain was isolated from a 17-year-old patient [11] that we suppose to be related to the acute PCM form. However, the lack of clinical information of this case occurred in 1970 does not allow us to conclude that *P. brasiliensis* PS2 can cause both clinical forms of the disease. Further, more studies must be done in order to clarify the clinical implications and response to treatment of this phylogenetic species of *P. brasiliensis*.

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Compliance with Ethical Standards

Conflicts of interests The authors declare that they have no competing interests.

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