Case Report: Fatal Fungemia due to *Paracoccidioides lutzii*

Rosane Christine Hahn, Anderson Messias Rodrigues, Cor Jesus Fernandes Fontes, Andreia Ferreira Nery, Tomoko Tadano, Luiz de Pádua Queiroz Júnior, and Zoilo Pires de Camargo*

Núcleo de Doenças Infecciosas e Tropicais, Universidade Federal do Mato Grosso (UFMT), Cuiabá, MT, Brazil; Universidade Federal de São Paulo (UNIFESP), Departamento de Microbiologia, Imunologia e Parasitologia, Disciplina de Biologia Celular, São Paulo, SP, Brazil

Abstract. We report the first case of fungemia caused by *Paracoccidioides lutzii* in a 51-year-old male farm worker from the central-west region of Brazil. The fungus was isolated from blood cultures and the species was confirmed by phylogenetic identification. Despite specific treatment and intensive care, the patient died 39 days after admission.

INTRODUCTION

Paracoccidioidomycosis (PCM) is a human systemic mycosis caused by different species of thermophilic fungi in the genus *Paracoccidioides*. It is assumed that the contamination of subjects is caused by inhalation of propagules from the environment,^{1,2} which leads to a primary pulmonary infection and then disseminates to other organs and systems. Two forms of the disease are distinguished: the acute (subacute) juvenile form and the chronic adult form. Secondary lesions appear frequently in the mucous membranes, lymph nodes, skin, and adrenals.¹ Occupations that predispose persons to infection include people involved in activities and exposure to contaminated soil, affecting mainly rural workers during their most productive years of life.

Recent studies revealed high genetic variability among isolates that were morphologically identified as *Paracoccidioides brasiliensis*,^{3,4} which has led to the recognition and introduction of new pathogenic species. The phylogenetic species embedded in the *P. brasiliensis* complex include S1 (species 1), PS2 (phylogenetic species 2), PS3 (phylogenetic species 3), and PS4 (phylogenetic species 4), whereas the clade harboring the isolate Pb01 is placed at a relatively large distance from the *P. brasiliensis* complex and currently is referred to as a new biological species *Paracoccidioides lutzii*, which is prevalent in the central-west region of Brazil.⁵

From both therapeutic and epidemiological perspectives, it is essential to identify the *Paracoccidioides* species. Serological methods based on the detection of circulating antibodies and antigens have been widely adopted for the immunodiagnosis and patients follow-up.⁶ However, in recent years, we observed a high number of false negative tests using the standard antigenic preparation derived from the fungus B-339 (ATCC 32069; PS3) in double immunodiffusion (DID) or enzyme-linked immunosorbent assay.^{7,8} This incongruence suggests an antigenic variability in the genus *Paracoccidioides* and may be related to subjects infected with different phylogenetic species. Here, we describe an atypical case of PCM fungemia, associated with negative serology in a male farm worker and discuss the importance of the early diagnosis for the management of the disease.

CASE REPORT

On January 31, 2012, a 51-year-old male farm worker living in the central-west region of Brazil (Indianapolis, MT) for roughly 30 years was referred by the public hospital in Barra do Garças, MT, for admission to the Júlio Muller University Hospital (HJUM/UFMT) in Cuiabá, MT, Brazil. The patient was a smoker (70 cigarettes per day) and an alcoholic but presented with no known comorbidities. Clinically, he presented with respiratory failure (RF = 48) and dullness in the middle one-third of the right hemithorax (RHT), with diffuse wheezing, snoring, and universal crackling, particularly in the RHT. The physical exam revealed intense hypoxemia (arterial blood gas PaO₂/FiO₂ = 180) and signs of chest muscle fatigue. Before orotracheal intubation, the patient reported dyspnea for the first time 6 months before admission. The dyspnea had progressively worsened with productive cough and episodes of hemoptysis, fever, wasting syndrome, and night sweats. No enlarged lymph nodes or oropharyngeal lesions were observed. The patient had sought medical care on numerous occasions but had not received a precise etiological diagnosis. When the respiratory and systemic symptoms worsened rapidly, the patient was admitted to the hospital and referred to the intensive care unit (ICU) for support and etiological investigation. During this period, the following therapeutic regimen was initiated immediately: 50 mg/day of amphotericin B deoxycholate, 4.5 g piperacillin/tazobactam every 6 hours, and 1 g vancomycin every 12 hours. Microscopic examination of tracheal aspirate at the time of admission revealed the presence of *Paracoccidioides* spp. yeast cells resembling a steering wheel, but the samples were negative for acid-fast bacilli and human immunodeficiency virus (HIV) serology was nonreactive. A chest radiograph showed bilateral, diffuse interstitial alveolar infiltrates (Figure 1). The patient exhibited only partial clinical–radiological improvement, despite ICU support, mechanical ventilation, and prolonged treatment with amphotericin B and other intravenous antimicrobials (600 mg linezolid every 12 hours, 2 g meropenem every 8 hours, and 500 U polymyxin B), which were gradually readjusted throughout the patient's stay in the ICU. Twenty-eight days later, blood samples that were collected upon admission and cultured on Sabouraud dextrose agar plates revealed the presence of *Paracoccidioides* spp. The first clue that we were dealing with an atypical *Paracoccidioides* strain was the serological results. Double immunodiffusion tests using antigenic preparation from the standard strain *P. brasiliensis* B-339⁹ generated negative results, whereas preparation using the autochthonous strain produced reactivity signals (Figure 2). Despite the specific prescribed treatment

* Address correspondence to Zoilo Pires de Camargo, Departamento de Microbiologia, Imunologia e Parasitologia, Disciplina de Biologia Celular, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil. 04023-062 Rua Botucatu, 862/8º andar. São Paulo, SP, Brazil. E-mail: zpcamargo@unifesp.br

FIGURE 1. Thoracic x-ray of patient with paracoccidioidomycosis caused by *Paracoccidioides lutzii* showing bilateral diffuse infiltrate.

and continuous intensive care support, the patient died 39 days after admission.

RESULTS

Blood culture isolates suspected of *Paracoccidioides* spp. were cultivated at 25°C on Fava-Netto's semi-solid medium¹⁰ and sub-cultured until a pure culture was obtained. The dimorphic nature of *Paracoccidioides* was shown by converting the fungus to yeast form at 37°C on Fava-Netto's semi-solid medium and the mycelial form at 25°C on potato dextrose agar medium (Difco Laboratories, Detroit, MI) (Figure 3). Genomic DNA was extracted and purified directly from fungal colonies using the Fast DNA kit (MP Biomedicals, Vista, CA) according to the manufacturer's instructions.

Suspected colonies were subjected to *HSP70* gene amplification using the primers HSPMMT1 and PLMMT1 as described by Teixeira and others⁴ to identify an exclusive indel region of *P. lutzii*. The clinical isolate (no. 9840) presented positive amplification similar to the amplicon found in isolate Pb01 (ATCC MYA-826, positive control) (Figure 4A). The molecular characterization of the mating type idiomorph was carried out by polymerase chain reaction (PCR) using the primers MAT1-1F and MAT1-1R targeting *MAT1-1* (α -box), and

FIGURE 2. Immunodiffusion test for paracoccidioidomycosis. In the well center, serum from patient 9840 (S); in the outer wells exoantigen from *Paracoccidioides brasiliensis* B-339 (AgPb339) and *Paracoccidioides lutzii* (AgPl).

FIGURE 3. *Paracoccidioides* spp. isolated from blood cultures. Macroscopic view of thermodimorphism at (A) 25°C in potato dextrose agar (PDA) medium and (B) 37°C in Fava-Netto's medium.

MAT1-2F and MAT1-2R targeting *MAT1-2* (HMG) as described by Teixeira and others.¹¹ Isolate 9840 is a heterothallic strain harboring the *MAT1-1* locus (Figure 4B and C).

Three loci were chosen for amplification and sequencing. The 43 kDa glycoprotein (*GP43*),¹² ADP-ribosylation factor (*ARF*),¹³ and α -tubulin (*TUB1*)¹³ loci were amplified directly from the genomic DNA by PCR as described previously.³ Amplified products were gel-purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI) following the manufacturer's instructions. The PCR fragments were completely sequenced in an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

The *GP43* exon-2, *ARF*, and *TUB1* nucleotide sequences from other isolates belonging to the *P. brasiliensis* complex were included as reference strains for the phylogenetic analysis. These sequences were previously deposited at GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) and described by Matute and others,³ Teixeira and others,⁴ and Marques-da-Silva and others.⁷ The nucleotide sequences were aligned using the Simultaneous Alignment and Tree Estimation software (SATé 2.2.2).¹⁴ The alignments were corrected manually to avoid mispaired bases. Evolutionary analyses were carried out in MEGA5¹⁵ for the combined data set using the maximum likelihood and neighbor-joining methods. Evolutionary distances were computed using the Kimura 2-parameter model with 1,000 bootstrap replicates.

The complete alignment included 81 taxa. The concatenated aligned sequences were 1,090 base pair (bp) long, including 982 invariable characters, 92 (8.44%) variable parsimony-informative sites, and 16 singletons. Isolate 9840 clustered in the *Paracoccidioides lutzii* clade with high bootstrap values, confirming the genotyping method based on *HSP70* amplification (Figure 5). The GenBank accession nos. for the partial ADP-ribosylation factor, *GP43* exon2, and *TUB1* genes are KC732776, KC732777, and KC732778, respectively.

DISCUSSION

Fungemia caused by dimorphic fungi in the order Onygenales are rare in clinical presentation; just a few cases have been reported^{16–19} and they are frequently associated with an immunosuppressed state, such as HIV infection.²⁰ Other underlying diseases may also be involved and increase the risk of fungemia in human patients.^{20,21}

FIGURE 4. Genotyping the *Paracoccidioides* spp. based on *HSP70* marker and MAT locus amplification. (A) Isolate 9840 presented positive *HSP70* amplification, similar to the positive control (Pb01). Polymerase chain reaction amplification of *MAT1-1* (B) and *MAT1-2* (C) idiomorphs.

To the best of our knowledge, this study is the first to report a case of fungemia caused by *P. lutzii* in an immunocompetent patient, which distinguishes our case from the classical risk group. The criteria used here to define the patient as immunocompetent was based on absence of any underlying disease such as HIV, cancer, tuberculosis, and hepatitis. However, he was alcoholic and presented wasting syndrome, which would be caused by the disseminated fungal infection and/or another non-diagnosed clinical condition. The mechanism by which extrapulmonary dissemination can lead to infection of the bloodstream and subsequent hematogenous dissemination is unknown. Pathogens may cross the blood-brain barrier transcellularly, paracellularly, and/or by infected phagocytes (so-called Trojan horse mechanism). Transcellular traversal of the blood-brain barrier has been shown for several bacterial pathogens^{22,23} and fungal pathogens, such as *Candida albicans*²⁴ and *Cryptococcus neoformans*.²⁵

For a century, the etiological agent of PCM has been attributed to the species *P. brasiliensis* s.l., which was previously assumed to be a monotypic taxon, although recent publications^{3,4} support the idea of several cryptic phylogenetically related species in a complex. The real incidence of each phylogenetic species and its implication on ecology and clinical practice is difficult to establish because of a lack of information in the literature in regards to the distribution of these entities. Species 1 (S1) is broadly distributed throughout Latin America^{3,4,26} and frequently recovered from environmental and clinical cases. Currently, phylogenetic species 3 (PS3) is restricted to clinical cases in Colombia,^{3,26} and just a few isolates from phylogenetic species 2 (PS2) have been reported in the literature^{3,26} from Brazil and Venezuela. The geographical epicenter of *P. lutzii* is the central-west region of Brazil,⁴ with scattered cases reported outside of this area^{7,26} and a single strain reported outside Brazil.⁴ Acquisition of the disease occurs through inhalation of conidia of *Paracoccidioides* spp. Therefore, the occupation and geographical area of housing were risk factors in our patient for the acquisition of PCM caused by *P. lutzii*.

The geographic areas of the phylogenetic entities in the genus *Paracoccidioides* have been indicated to overlap. Recently, Arantes and others²⁷ reported environmental nucleic acid sequences belonging to *P. lutzii* in the hyperendemic region of Botucatu in the southeast region of Brazil,²⁷ an area formerly assumed to have a high prevalence of species S1.²⁻⁴ However, no increase in clinical cases of PCM caused by *P. lutzii* has been seen in areas others than the central-west region of Brazil, which could be related to differences in the survival and growth of this species in nature, differences in human exposure, or even host

resistance to infection in this area, and the misidentification of this pathogenic species by currently available techniques.

Appropriate treatment depends on accurate diagnosis. The gold standard for mycological diagnosis, including PCM, is the isolation of the etiological agent in culture. However, for the isolation of clinical specimens in PCM, the appearance of the first colonies could take months and delay diagnosis. On the other hand, PCM serology has evolved over the years²⁸ and been reported to be an essential tool for rapid diagnosis^{29,30} and patient follow-up.^{6,31} Unfortunately, from a seroepidemiological point of view, the diagnosis of PCM caused by divergent phylogenetic species is difficult based on antigenic preparations of the reference strain B-339 (PS3),^{7,8} and none of the serological tests developed to date are able to clearly diagnosis PCM caused by the five cryptic species, which favors delayed diagnosis and false negative results. Our negative results for the double immunodiffusion test using the antigenic preparation of the B-339 strain are in agreement with previous studies,^{7,8} which reinforces the idea of antigenic variability in the genus *Paracoccidioides*. The negative serology should not be interpreted only as the absence of antibodies caused by immunosuppression³² or drug therapy³¹; thus, we emphasize the need for specific antigenic preparation from autochthonous strains, in addition to the use of the standard preparation of B-339, to improve the sensitivity and specificity of the serological test. We hypothesized that several cases of PCM caused by different phylogenetic species in the genus *Paracoccidioides* may have been underestimated in Latin America when only preparations derived from the standard strain B-339 were used.

Studies evaluating the clinical aspects of the PCM patients and the phylogenetic species must clarify whether there is any correlation between the etiological agent and the clinical manifestation or distinct susceptibility profiles. However, it remains unclear whether there is a correlation between drug susceptibility and clinical outcome in PCM caused by *P. brasiliensis* or *P. lutzii*.³³ The outcome of fungemia depends on several factors, but earlier diagnosis and treatment may decrease the risk of mortality. Accurate identification of the etiological agent of PCM is important for choosing the best therapeutic method. For the treatment of endemic mycosis, including PCM, itraconazole is the best choice,^{34,35} but the use of alternative drugs, including sulfonamides, alone or in combination (sulfamethoxazole and trimethoprim),^{7,36} is frequent in some areas because of its availability from public health services. Notably, empirical treatment of tuberculosis or other pulmonary disorders may decrease the chances of healing and culminate in worsening clinical symptoms.

FIGURE 5. Phylogenetic analysis using the maximum likelihood method based on the concatenate data set of ADP-ribosylation factor, *GP43* exon 2, and *TUB1* genes. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (NJ/ML). The evolutionary distances were computed using the Kimura 2-parameter method. All positions containing gaps and missing data were eliminated.

In conclusion, we report an atypical case of PCM caused by *P. lutzii* in central-western Brazil. The patient did not present any evidence of immunosuppression and progressed rapidly, dying 39 days after admission and not responding to classical drug administration. Early diagnosis and proper treatment can result in the healing of patients who develop atypical cases.

Received August 20, 2013. Accepted for publication April 14, 2014.

Published online May 12, 2014.

Financial support: A. M. Rodrigues is a fellow and received financial support from the Fundação de Amparo à Pesquisa do Estado de São

Paulo (FAPESP, 2011/07350-1). Z. P. de Camargo also received financial support from FAPESP (2009/54024-2).

Authors' addresses: Rosane Christine Hahn, Cor Jesus Fernandes Fontes, Andreia Ferreira Nery, Tomoko Tadano, and Luiz de Pádua Queiroz Júnior, Núcleo de Doenças Infecciosas e Tropicais, Universidade Federal do Mato Grosso, MT, Brasil, E-mails: rchahn@terra.com.br, corfontes@gmail.com, andreia_nery@yahoo.com.br, mokacba@terra.com.br, and luizpadua.biomedico@hotmail.com. Anderson Messias Rodrigues and Zoilo Pires de Camargo, Universidade Federal de São Paulo, Departamento de Microbiologia, Imunologia e Parasitologia, Disciplina de Biologia Celular, São Paulo, Brasil, E-mails: amrodrigues@unifesp.br and zpcamargo@unifesp.br.

REFERENCES

- Brummer E, Castañeda E, Restrepo A, 1993. Paracoccidioidomycosis: an update. *Clin Microbiol Rev* 6: 89–117.
- Barrozo LV, Benard G, Silva ME, Bagagli E, Marques SA, Mendes RP, 2010. First description of a cluster of acute/sub-acute paracoccidioidomycosis cases and its association with a climatic anomaly. *PLoS Negl Trop Dis* 4: e643.
- Matute DR, McEwen JG, Puccia R, Montes BA, San-Blas G, Bagagli E, Rauscher JT, Restrepo A, Morais F, Niño-Vega G, Taylor JW, 2006. Cryptic speciation and recombination in the fungus *Paracoccidioides brasiliensis* as revealed by gene genealogies. *Mol Biol Evol* 23: 65–73.
- Teixeira MM, Theodoro RC, de Carvalho MJ, Fernandes L, Paes HC, Hahn RC, Mendoza L, Bagagli E, San-Blas G, Felipe MS, 2009. Phylogenetic analysis reveals a high level of speciation in the *Paracoccidioides* genus. *Mol Phylogenet Evol* 52: 273–283.
- Teixeira MM, Theodoro RC, Oliveira FF, Machado GC, Hahn RC, Bagagli E, San-Blas G, Felipe MS, 2013. *Paracoccidioides lutzii* sp. nov.: biological and clinical implications. *Med Mycol* 52: 19–28.
- Marques da Silva SH, de Mattos Grosso D, Lopes JD, Colombo AL, Blotta MH, Queiroz-Telles F, Pires de Camargo Z, 2004. Detection of *Paracoccidioides brasiliensis* gp70 circulating antigen and follow-up of patients undergoing antimycotic therapy. *J Clin Microbiol* 42: 4480–4486.
- Marques-da-Silva SH, Rodrigues AM, de Hoog GS, Silveira-Gomes F, de Camargo ZP, 2012. Occurrence of *Paracoccidioides lutzii* in the Amazon region: description of two cases. *Am J Trop Med Hyg* 87: 710–714.
- Batista J Jr, de Camargo ZP, Fernandes GF, Vicentini AP, Fontes CJ, Hahn RC, 2010. Is the geographical origin of a *Paracoccidioides brasiliensis* isolate important for antigen production for regional diagnosis of paracoccidioidomycosis? *Mycoses* 53: 176–180.
- Camargo ZP, Berzaghi R, Amaral CC, Silva SH, 2003. Simplified method for producing *Paracoccidioides brasiliensis* exo-antigens for use in immunodiffusion tests. *Med Mycol* 41: 539–542.
- Fava-Netto C, Vegas VS, Sciannamea IM, Guarnieri DB, 1969. The polysaccharide antigen from *Paracoccidioides brasiliensis*. Study of the time of cultivation necessary for the preparation of the antigen. *Rev Inst Med Trop Sao Paulo* 11: 177–181.
- Teixeira Md M, Theodoro RC, Derengowski Ld S, Nicola AM, Bagagli E, Felipe MS, 2013. Molecular and morphological data support the existence of a sexual cycle in species of the genus *Paracoccidioides*. *Eukaryot Cell* 12: 380–389.
- Cisalpino PS, Puccia R, Yamauchi LM, Cano MI, da Silveira JF, Travassos LR, 1996. Cloning, characterization, and epitope expression of the major diagnostic antigen of *Paracoccidioides brasiliensis*. *J Biol Chem* 271: 4553–4560.
- Kasuga T, White TJ, Taylor JW, 2002. Estimation of nucleotide substitution rates in Eurotiomycete fungi. *Mol Biol Evol* 19: 2318–2324.
- Liu K, Warnow TJ, Holder MT, Nelesen SM, Yu J, Stamatakis AP, Linder CR, 2012. SATé-II: Very fast and accurate simultaneous estimation of multiple sequence alignments and phylogenetic trees. *Syst Biol* 61: 90–106.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731–2739.
- Hadad DJ, Pieres Mde F, Petry TC, Orozco SF, Melhem Mde S, Paes RA, Gianini MJ, 1992. *Paracoccidioides brasiliensis* (Lutz, 1908) isolated by hemoculture in a patient with the acquired immunodeficiency syndrome (AIDS). *Rev Inst Med Trop Sao Paulo* 34: 565–567.
- Tan G, Kaufman L, Peterson EM, de la Maza LM, 1993. Disseminated atypical blastomycosis in two patients with AIDS. *Clin Infect Dis* 16: 107–111.
- Rempe S, Sachdev MS, Bhakta R, Pineda-Roman M, Vaz A, Carlson RW, 2007. *Coccidioides immitis* fungemia: clinical features and survival in 33 adult patients. *Heart Lung* 36: 64–71.
- Assi MA, Sandid MS, Baddour LM, Roberts GD, Walker RC, 2010. Risk factor analysis of *Histoplasma capsulatum* fungemia. *Med Mycol* 48: 85–89.
- Gonzalez CE, Venzon D, Lee S, Mueller BU, Pizzo PA, Walsh TJ, 1996. Risk factors for fungemia in children infected with human immunodeficiency virus: a case-control study. *Clin Infect Dis* 23: 515–521.
- Reimer LG, Wilson ML, Weinstein MP, 1997. Update on detection of bacteremia and fungemia. *Clin Microbiol Rev* 10: 444–465.
- Unkmeir A, Latsch K, Dietrich G, Wintermeyer E, Schinke B, Schwender S, Kim KS, Eigenthaler M, Frosch M, 2002. Fibronectin mediates Opc-dependent internalization of *Neisseria meningitidis* in human brain microvascular endothelial cells. *Mol Microbiol* 46: 933–946.
- Kim KS, 2002. Strategy of *Escherichia coli* for crossing the blood-brain barrier. *J Infect Dis* 186: S220–S224.
- Jong AY, Stins MF, Huang S-H, Chen SH, Kim KS, 2001. Traversal of *Candida albicans* across human blood-brain barrier *in vitro*. *Infect Immun* 69: 4536–4544.
- Chang YC, Stins MF, McCaffery MJ, Miller GF, Pare DR, Dam T, Paul-Satyasee M, Kim KS, Kwon-Chung KJ, 2004. Cryptococcal yeast cells invade the central nervous system via transcellular penetration of the blood-brain barrier. *Infect Immun* 72: 4985–4995.
- Theodoro RC, Teixeira Md M, Felipe MS, Paduan Kd S, Ribolla PM, San-Blas G, Bagagli E, 2012. Genus *Paracoccidioides*: species recognition and biogeographic aspects. *PLoS ONE* 7: e37694.
- Arantes TD, Theodoro RC, Da Graça Macoris SA, Bagagli E, 2013. Detection of *Paracoccidioides* spp. in environmental aerosol samples. *Med Mycol* 51: 83–92.
- Camargo ZP, 2008. Serology of paracoccidioidomycosis. *Mycopathologia* 165: 289–302.
- Marques da Silva SH, Colombo AL, Blotta MH, Lopes JD, Queiroz-Telles F, Pires de Camargo Z, 2003. Detection of circulating gp43 antigen in serum, cerebrospinal fluid, and bronchoalveolar lavage fluid of patients with paracoccidioidomycosis. *J Clin Microbiol* 41: 3675–3680.
- Silveira-Gomes F, Sarmiento DN, Pinto TM, Pimentel RF, Nepomuceno LB, Espírito Santo EP, Mesquita-da-Costa M, Camargo ZP, Marques-da-Silva SH, 2011. Development and evaluation of a latex agglutination test for the serodiagnosis of paracoccidioidomycosis. *Clin Vaccine Immunol* 18: 604–608.
- Marques da Silva SH, Queiroz-Telles F, Colombo AL, Blotta MH, Lopes JD, Pires de Camargo Z, 2004. Monitoring gp43 antigenemia in paracoccidioidomycosis patients during therapy. *J Clin Microbiol* 42: 2419–2424.
- Marques SA, Robles AM, Tortorano AM, Tuculet MA, Negroni R, Mendes RP, 2000. Mycoses associated with AIDS in the Third World. *Med Mycol* 38 (Suppl 1): 269–279.
- Cruz RC, Werneck SM, Oliveira CS, Santos PC, Soares BM, Santos DA, Cisalpino PS, 2013. Influence of different media, incubation times, and temperatures for determining the MICs of seven antifungal agents against *Paracoccidioides brasiliensis* by microdilution. *J Clin Microbiol* 51: 436–443.
- Shikanai-Yasuda MA, Telles Filho Fde Q, Mendes RP, Colombo AL, Moretti ML, 2006. Guidelines in paracoccidioidomycosis. *Rev Soc Bras Med Trop* 39: 297–310.
- Queiroz-Telles F, Goldani LZ, Schlamm HT, Goodrich JM, Ingroff AE, Yasuda MAS, 2007. An open-label comparative pilot study of oral voriconazole and itraconazole for long-term treatment of paracoccidioidomycosis. *Clin Infect Dis* 45: 1462–1469.
- Hahn RC, Morato Conceição YT, Santos NL, Ferreira JF, Hamdan JS, 2003. Disseminated paracoccidioidomycosis: correlation between clinical and *in vitro* resistance to ketoconazole and trimethoprim sulfamethoxazole. *Mycoses* 46: 342–347.