

## Implementation of a Training Course Increased the Diagnosis of Histoplasmosis in Colombia

Diego H. Caceres, Alejandra Zuluaga, Karen Arango-Bustamante, Catalina de Bedout, Ángela María Tobón, Ángela Restrepo, Beatriz L. Gómez, Luz Elena Cano, and Ángel González\*

Medical and Experimental Mycology Group, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia; School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia; School of Microbiology, Universidad de Antioquia, Medellín, Colombia; Basic and Applied Microbiology Research Group (MICROBA), School of Microbiology, Universidad de Antioquia, Medellín, Colombia

**Abstract.** Histoplasmosis causes a significant mortality, especially persons living with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) from developing countries where access to both appropriate diagnostic methods and antiretroviral therapy are limited. A total of 81 physicians assigned to 17 Colombian departments (states) received training in the clinical, epidemiological, and diagnostic aspects of histoplasmosis. Once this training was received and during the period of October 2009–November 2012, these physicians sent biological samples for immunodiagnostic, mycological, and molecular tests from their patients with suspicion of histoplasmosis. A total of 1,536 samples from 768 patients were evaluated. Of the 768 patients studied, 463 (60%) were HIV positive, 214 (28%) HIV negative, and in 91 (12%) this diagnosis was unknown, and 538 (70%) were males. The 1,536 specimens studied comprised 722 sera, 439 blood samples, and 241 urines, which were tested by immunodiffusion (ID), culture, and antigenuria, respectively; in addition, 134 specimens were tested by performing a molecular assay. Histoplasmosis was diagnosed in 133 patients (17%). After the training, we observed more diagnoses from 27 to 44 cases per year. In this study, a significantly increased number of histoplasmosis cases reported by year were observed after implementing an educational training program.

### INTRODUCTION

Histoplasmosis is frequently diagnosed in the Americas and some regions of Asia and Africa albeit with decreased rates in the last two areas.<sup>1,2</sup> The clinical spectrum of this disease is variable, and the presentation often depends on various factors including the concentration of the infectious inoculum, the host's structural or immunologic abnormalities at the time of infection, and the organs involved.<sup>3,4</sup> Some populations appear to be more susceptible to this disease than others, such as individuals at the extreme ages of life, those undergoing immunosuppressive therapy, solid organ recipients, and, especially, individuals infected with the human immunodeficiency virus (HIV) having CD4<sup>+</sup> lymphocyte counts less than 150/ $\mu$ L.<sup>5</sup>

The incidence of the histoplasmosis in the United States varies from 2% to 5% in persons with HIV/acquired immunodeficiency syndrome (AIDS), but in highly endemic regions this incidence may be as high as 27%.<sup>4</sup> Despite the advances in the treatment of individuals with HIV/AIDS, histoplasmosis represents an important public health problem in resource-limited settings where the mortality associated with this fungal infection is high, ranging from 13% to 48%.<sup>6</sup>

Diagnosis of this fungal disease includes culture, histopathological analysis, and immunodiagnostic and molecular tests that present variations in their sensitivity and specificity values.<sup>7,8</sup> The diagnosis of this fungal disease is a continuous challenge, especially in resource-limited regions where this disease is endemic and where there is a high frequency of AIDS. Thus, establishment of an early diagnosis of this disease is essential to reduce morbidity and mortality. Unfortunately, in some regions of Latin America, a limited number of diagnostic tests are available.<sup>9</sup> Previous reports emphasize the need to strengthen the ability to perform a timely and

appropriate diagnosis, and subsequently, gain access to specific antifungal treatment.<sup>9,10</sup>

In 1971, in a large study done in five regions of Colombia, Carmona reported an average of 22% positive reactors to histoplasmin suggesting that a significant proportion of the native population had been in contact with the fungus.<sup>11</sup> In 2011, Arango and others identified a total of 434 patients with histoplasmosis diagnosed from 1992 to 2008 with AIDS patients accounting for 70% of all cases. Diagnosis was made by microscopic observation of *Histoplasma capsulatum* in 50% patients, by culture in 58% and by serological tests in 15% of the cases.<sup>12</sup> More recently, Arango-Bustamante and others<sup>13</sup> determined the value of culture and serological tests in the diagnosis of histoplasmosis; in this report, a total of 391 patients were analyzed and their results indicated that culture was the most reliable fungal diagnostic method for the HIV/AIDS patient population suffering from this mycosis, and contrary to what is generally believed, serological assays were useful for diagnosing histoplasmosis in these patients.

The aim of this study was to evaluate if implementation of a specific training course could impact the diagnosis of histoplasmosis in Colombian populations.

### MATERIALS AND METHODS

**Study design.** We conducted a prospective surveillance from October 2009 to November 2012 among individuals with clinically suspected histoplasmosis originating in 17 Colombian political divisions (departments). Diagnosis of histoplasmosis was made on the basis of the recommendations of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG).<sup>14</sup>

**Implementation of a medical educational program for the diagnosis of histoplasmosis.** A total of 81 physicians specialized in internal medicine and infectious diseases, as well as medical residents ascribed to different Colombian departments (states) where the program had been implemented,

\* Address correspondence to Ángel González, School of Microbiology, Universidad de Antioquia, Calle 70 No. 52-51, Medellín, Colombia. E-mail: angel.gonzalez@udea.edu.co

were trained in the facilities of the Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia. This training course had an intensity of 8 hours (1 day) and covered different aspects of the histoplasmosis including clinical, epidemiological, diagnostic, and treatment aspects. The training course, consisting of workshops and lectures, was taught by experts on medical mycology and on the clinical and epidemiological manifestations of histoplasmosis (S1). Personnel of the Medical and Experimental Mycology Unit at the CIB analyzed all biological samples evaluated during the implementation of this program. The program consisted of two stages, the first from October 2009 to October 2011, when the physicians submitted specimens from patients with clinically suspected histoplasmosis. Clinical specimens included sera for the immunodiffusion test (ID), blood for cultures, and tissues as well as biological fluids for a nested polymerase chain reaction (PCR) for *H. capsulatum* (Hc100-PCR). The second stage of the program was conducted from November 2011 to November 2012; in this period, the physicians submitted sera for ID and urine samples for performance of the antigenuria test (Hc-Ag). Attendance of the physicians from different Colombian states to the course as well as the cost of testing and shipping of the samples were covered by a grant assigned to this particular research project.

**Case definition.** According to the EORTC, a case of histoplasmosis was considered to be proven if the etiologic agent (*H. capsulatum*) could be isolated from any of the following samples: blood, tissue, sterile fluids, or respiratory specimens, or if the histopathological analyses showed the presence of intracellular yeast. A case was considered probable if *H. capsulatum* could not be isolated, but there was serological evidence of infection, such as the presence of either H or M band or both bands by ID or a positive Hc-Ag test from urine. Although molecular tests have not been yet considered a diagnostic tool for this mycosis, in this study we evaluated a nested PCR (Hc100-PCR) previously validated in our laboratory.<sup>15</sup>

**Laboratory tests.** *Serology.* The ID and complement fixation (CF) techniques were performed according to standardized protocols previously described.<sup>16–18</sup>

*Blood culture.* Blood samples were processed using a fully automated method (BACTEC™ Myco/FLytic, catalog no. 442003; BD, Franklin Lakes, NJ). Positive blood cultures were subcultured on plates with Mycosel™ (catalog no. 295698; BD) and Sabouraud Dextrose agar™ (catalog no. 221988; BD) to isolate the etiologic agent.

*Nested PCR for H. capsulatum (Hc100-PCR).* Specific primers targeting the gene coding for a 100-kDa protein of *H. capsulatum* were used. This assay was performed employing biopsies, sterile body fluids, and samples fixed in paraffin, following the standardized procedures described by Muñoz and others.<sup>15</sup> All the biological samples tested, with the exception of those that were formalin-fixed paraffin-embedded (FFPE), were also cultured on plates with Mycosel (BD) and Sabouraud Dextrose agar (BD).

*Antigenuria (Hc-Ag).* An enzyme-linked immunosorbent assay (ELISA) antigen detection test using a polyclonal antibody that recognized an *H. capsulatum* polysaccharide antigen (HPA) was used and performed as described elsewhere.<sup>19</sup>

*Ethics.* All participants provided an informed consent before participating in this study guaranteeing anonymity.

*Data collection and statistical analysis.* Patients' data were collected using a standardized questionnaire consisting of

demographical, clinical, and laboratory variables; the information collected was stored in a Microsoft Excel file (Redmond, WA). Absolute and relative frequencies were calculated. We used the  $\chi^2$  test to identify association between qualitative variables, and Student *t* test or Mann–Whitney *U* test to identify differences in averages or medians for quantitative variables. Statistically significant associations or differences were defined with a *P* value < 0.05. All analyses were performed using the software EPIDAT 3.1 (Galicia, Spain) and STATA 8.0 (College Station, TX).

## RESULTS

A total of 768 patients with clinically suspected histoplasmosis were evaluated. Of these, 538 (70%) were males and 230 (30%) females. The median age of patients was 37 years (0–91 years). HIV infection was present in 463 patients (60%), 214 patients (28%) were negative for this infection, and in 91 patients (12%) this diagnosis was unknown (Figure 1). HIV infection was also the most frequent risk factor reported in this cohort as 106 of the 463 patients (23%) were severely immunocompromised with a median CD4<sup>+</sup> T-cell count of 54 cell/μL (0–794 cell/μL) and 83 presented a median HIV viral load of 54,095 copies (< 40–10,000,000 copies).

Diagnosis of histoplasmosis was defined in 133 of the 768 (17%) patients with clinically suspected histoplasmosis (38 proven and 95 probable cases); this diagnosis was done using the available battery of laboratory tests (Figure 2). HIV infection was present in 105 of the 133 (79%) patients with the fungal disease (Figure 1). A marked difference in the sex ratio between male and female patients (3:1) with HIV was observed, whereas in HIV-negative patients this ratio was 1:1.

Regarding the geographical distribution of the places where histoplasmosis patients were diagnosed, the Department of Cundinamarca reported the highest number of patients with this fungal infection (26%; 34/133), followed by Santander (15%; 20/133), Quindío and Valle del Cauca with equal number of patients (14%; 19/133), Antioquia (10%; 14/133), Atlántico (8%; 11/133), Huila (6%; 8/133), and, finally, Caldas, Cesar, Meta, and Bolívar with a lower number of cases, less than 2%. Table 1 presents the number of patients

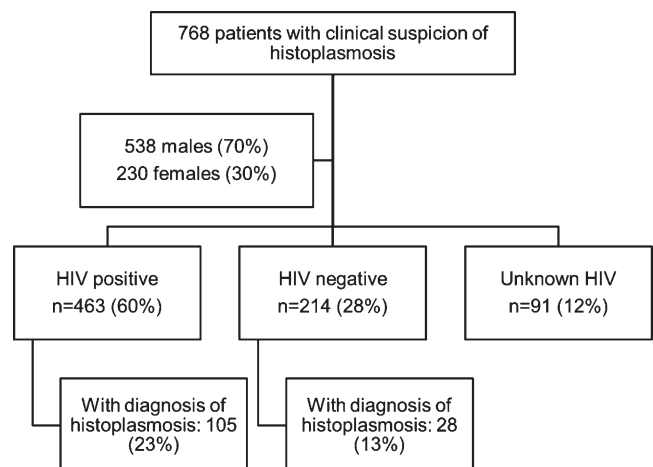


FIGURE 1. Flow chart for the diagnosis of patients with clinically suspected histoplasmosis in this program.

TABLE 1  
Colombian departments where histoplasmosis was diagnosed

State	No. of patients referred	No. of patients diagnosed with histoplasmosis (%)
Cundinamarca	211	34 (26)
Santander	83	20 (15)
Quindío	88	19 (14)
Valla del Cauca	113	19 (14)
Antioquia	81	14 (10)
Atlántico	93	11 (8)
Huila	53	8 (6)
Caldas	8	3 (2)
Cesar	5	2 (2)
Meta	17	2 (2)
Bolívar	5	1 (1)
Tolima	1	0 (0)
Norte de Santander	3	0 (0)
Cauca	3	0 (0)
Chocó	1	0 (0)
Sucre	1	0 (0)
Magdalena	2	0 (0)
Total	768	133 (100)

with clinically suspected histoplasmosis coming from different Colombian states.

Of the 1,536 specimens submitted by physicians, 722 were serum samples evaluated by ID, 439 were from blood cultures, 134 from tissues and other body fluids (32 biopsies from different tissues, 71 respiratory samples, 19 from sterile body fluids, and 12 bone marrow specimens). These were subjected to molecular testing using the Hc100-PCR. In addition, 241 urine samples were tested by ELISA to detect *Histoplasma* antigens.

The ID test proved reactive in 80 of 722 sera (11%); of note, in the HIV-infected patients a reactivity of 15% (67/435) was recorded in comparison with the HIV-negative patients in whom this reactivity was lower, 6% (13/206). In the remaining 81 patients with HIV unknown status, the ID test was negative. Regarding the CF test, titers greater than 1:32 were determined in 40 of the 80 patients diagnosed using the ID test (50%); this serological test exhibited titers greater than 1:32 in 35 (53%) of the 66 histoplasmosis-HIV patients.

*H. capsulatum* was isolated from 30 of the 439 (7%) blood cultures, with 29 of them corresponding to HIV-infected patients. The Hc100-PCR assay was reactive in 26 (19%) of the 134 samples referred for this assay, with 18 of them coming from HIV-positive patients. It is important to note that from 26 patients positive by the Hc100-PCR assay, 14 had another positive diagnostic test (ID, blood culture, histopathological, or Hc-Ag). Hc-Ag tests were positive in 39 of the 241 (16%) urine samples tested, with 31 of them corresponding to HIV-infected patients. These findings are described in Table 2.

A single laboratory test established the diagnosis of the mycosis in 93 cases. Thus, the ID allowed the diagnosis in 46 individuals; the Hc-Ag detection established the diagnosis in 24 patients, and the Hc100-PCR and blood culture allowed the diagnosis in 12 and 11 cases, respectively. Higher positivity in the remaining laboratory assays was more frequently recorded in the HIV patient population (34 patients) than in the HIV-negative individuals (two patients). Reactivity in the ID test was frequently accompanied by another positive assay (Figure 3).

TABLE 2

Positivity of the laboratory methods used according to the HIV status

Test/positivity	HIV positive	HIV negative	P value*
ID (N = 722)	N = 435	N = 206	0.001
Positive n (%)	67 (15)	13 (6)	
Blood culture (N = 439)	N = 279	N = 117	0.002
Positive n (%)	29 (10)	1 (1)	
Hc100-PCR (N = 134)	N = 76	N = 48	0.478
Positive n (%)	18 (24)	8 (17)	
Hc-Ag (N = 241)	N = 154	N = 53	0.545
Positive n (%)	31 (20)	8 (15)	

Hc-Ag = *Histoplasma capsulatum* antigenuria test; Hc100-PCR = nested polymerase chain reaction for *Histoplasma capsulatum*; HIV = human immunodeficiency virus; ID = immunodiffusion.

In patients without data concerning HIV infection (N = 91), none were positive in tests for histoplasmosis.

\* $\chi^2$  test for comparison between HIV-infected and non-HIV infected patients.

In addition, when the ID test also used antigens from other fungi, seven cases of aspergillosis and seven cases of paracoccidioidomycosis were detected; in addition, 11 patients had positive blood culture for other pathogens including *Cryptococcus neoformans* in three patients, *Candida* spp. in two, and *Mycobacterium* spp. in six (data not shown).

## DISCUSSION

This report presents findings resulting from the first educational program in Colombia focused on improving the diagnosis of histoplasmosis. The impact after the implementation of a training course in the diagnosis of this fungal disease is evaluated here. After teaching different aspects of this mycosis, 768 patients with clinically suspected histoplasmosis were enrolled in the study. Furthermore, a significant and substantial increase in the diagnosis of the mycosis in Colombia was noticed. Thus, compared with the results published by Arango and others, the number of histoplasmosis cases increased markedly from 27 to 44 cases per year; in addition, in departments where histoplasmosis had not been reported before (Huila and Cesar) the disease was identified, and changes were noticed in the number of cases reported by those departments with previous reports.<sup>12</sup> In a previous study done by our group, we found that during a period of 20 years (1987–2007), a total of 391 patients with the mycosis were diagnosed.<sup>13</sup> Noteworthy, in this study, after having taught the course, 133 patients with histoplasmosis were diagnosed in a period of only 3 years. These results clearly indicate that, after designing and implementing an educational training program that included gaining access to facilities and specialized laboratory methods, there was an increase in the number of patients detected with histoplasmosis. Similar experiences for other important diseases after educational programs for medical staff have also been shown to have an impact not only on diagnosis but also on control and prevention. As an example, a marked reduction of congenital transmission of toxoplasmosis in Brazil, and a significant reduction of community-associated methicillin-resistant *Staphylococcus aureus* outbreaks in Canada have been reported.<sup>20,21</sup> In developing countries from Asia and Africa, training courses in laboratory management and epidemiology have strengthened the technical laboratory capacity and the acquisition of new skills by the medical staff as a result of implementing educational programs for prevention and early detection of cancer and other diseases. A total of 92 outbreak investigations, 47 surveillance evaluations, 19 planned studies, and analyses of over

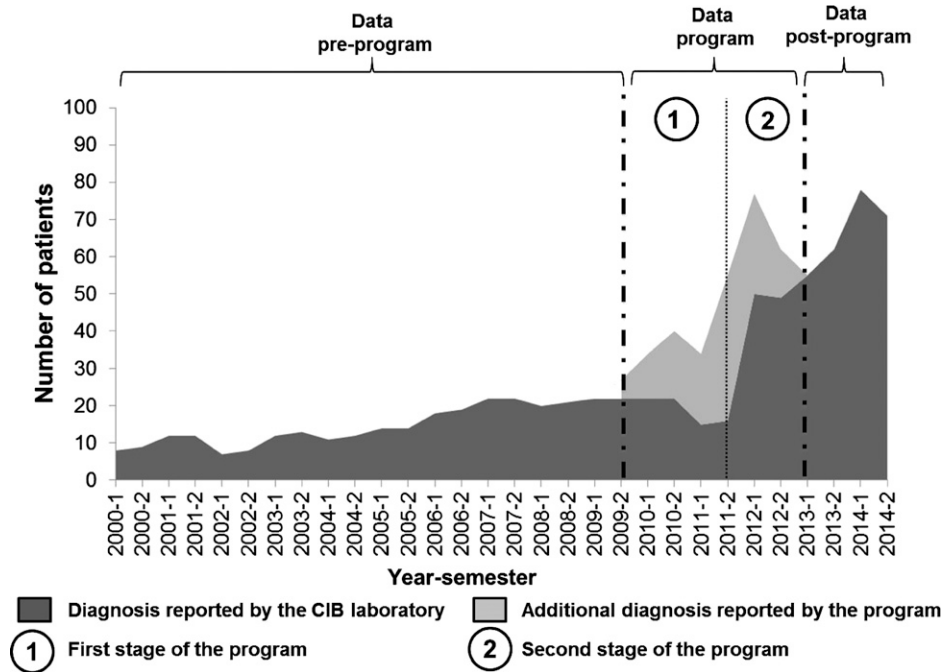


FIGURE 2. Impact of the educational program in the diagnosis of histoplasmosis: pre-, during, and post-program data comparison. Diagnosis of histoplasmosis reported from 2000 to 2014. Black areas in the figure represent the number of cases reported by a laboratory specialized in mycology while the gray areas represent the additional diagnoses of histoplasmosis established during the program (2009–2012). After the training, more diagnoses from 27 to 44 cases per year were recorded.

37 large databases have led to more than 56 articles presented at local and international conferences.<sup>22–27</sup> All these findings show that medical education programs, as well as the improvement of technical laboratory capacity, have a direct impact on the principal public health indicators.

On the other hand, in this study, it was confirmed that HIV infection was the most frequent risk factor for the appearance of histoplasmosis in the population studied; thus, the frequency of histoplasmosis in this group was 23% (105/463), compared with the group of patients without HIV where the

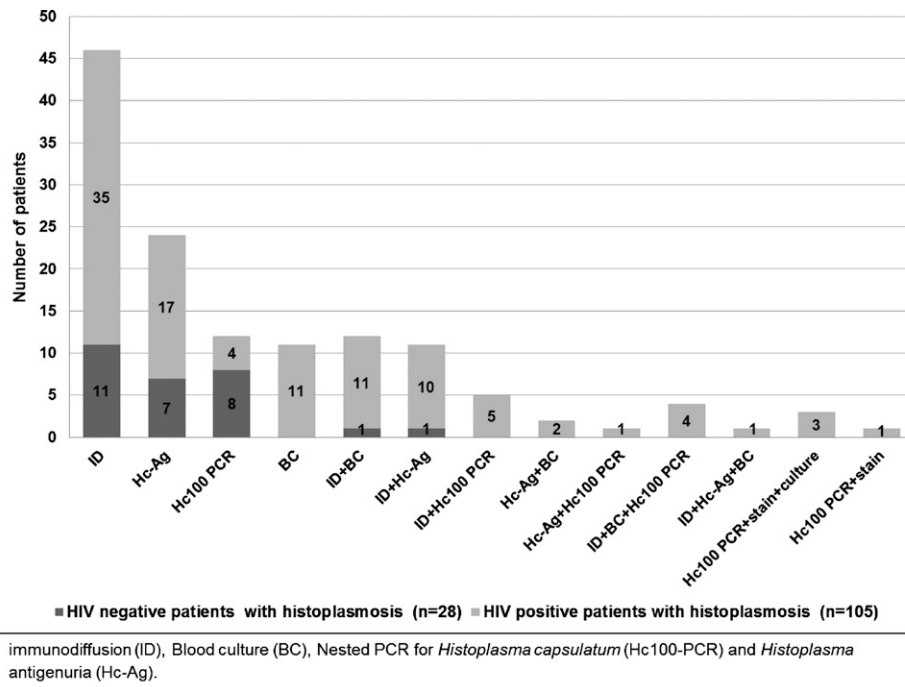


FIGURE 3. Diagnosis of histoplasmosis using a single laboratory assay or a combination of two or more laboratory tests.



frequency was 13% (28/214) ( $P < 0.001$ ). In Colombia and worldwide, HIV/AIDS is an important public health problem; the last official reports indicate that during the 2014 (first 14 epidemiological weeks), 2,129 new cases of HIV/AIDS had been reported.<sup>28</sup> In Colombia, the higher number of HIV/AIDS cases were diagnosed mainly in those departments where the program had been implemented, and consequently physicians should suspect histoplasmosis in this group of patients.<sup>29</sup> Another consideration to keep in mind is the increased population of patients with other risk factors, such as transplant recipients and patients subjected to immunosuppressed therapies, who may also develop histoplasmosis, so such risk factors should be taken into account when suspecting the mycosis.

Regarding laboratory tests, the ID and the Hc-Ag test, which showed high sensitivity in diagnosing histoplasmosis, are methods that do not require invasive procedures for specimen collection and that facilitate more rapid access to results. Nested PCR showed high sensitivity and specificity values; although this assay is not formally considered a diagnostic test, we have previously validated it.<sup>15</sup> Blood cultures presented difficulties concerning their capacity to diagnose histoplasmosis, especially in HIV-negative patients but not in HIV-positive patients, in whom the sensitivity of this test was higher. More generally, it was observed that 11 of 133 (8%) diagnosed cases reported in this study were achieved using only blood cultures. Of notice, we observed that the combination of diagnostic tests is the best strategy to reliably detect the highest number of histoplasmosis cases. Although this program could not be implemented in the whole Colombian territory, it was developed in the principal urban centers of the country, which hold a larger part of the Colombia's population able to receive medical services.

In conclusion, the medical training course implemented here showed a positive impact on the diagnosis of histoplasmosis in Colombia by allowing detection and diagnosis of more patients. In addition, it served to strengthen the laboratories capacity to perform tests that are adequate for the diagnosis of this mycosis. Finally, the access to more sensitive and rapid laboratory tests, such as Hc-Ag and molecular assays, could impact positively the diagnosis of this mycosis not only in Colombia but also in other countries worldwide.

Received February 6, 2015. Accepted for publication May 2, 2015.

Published online June 29, 2015.

Note: Supplemental information appears at [www.ajtmh.org](http://www.ajtmh.org).

Acknowledgments: We thank all the physicians and staff in health institutions from different Colombian states who participated in this study. We also express our appreciation to the expert instructors who joined us in the different training courses taught during this medical educational program.

Financial support: This work was supported by Strategy for Sustainability (2013–2014) for Research Groups, University of Antioquia (CODI).

Authors' addresses: Diego H. Caceres, Alejandra Zuluaga, Karen Arango-Bustamante, Catalina de Bedout, Ángela María Tobón, Ángela Restrepo, and Luz Elena Cano, Medical and Experimental Mycology Group, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia, E-mails: [diegocaceres84@gmail.com](mailto:diegocaceres84@gmail.com), [zuluaga.alejandra@gmail.com](mailto:zuluaga.alejandra@gmail.com), [karen.arango@gmail.com](mailto:karen.arango@gmail.com), [catybedout@gmail.com](mailto:catybedout@gmail.com), [atobon@cib.org.co](mailto:atobon@cib.org.co), [angelares@une.net.co](mailto:angelares@une.net.co), and [lcano@cib.org.co](mailto:lcano@cib.org.co). Beatriz L. Gómez, School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia, and Medical and Experimental Mycology Group, Corporación para Investigaciones

Biológicas (CIB), Medellín, Colombia, E-mail: [beatrizgomez@hotmail.com](mailto:beatrizgomez@hotmail.com). Ángel González, School of Microbiology, Universidad de Antioquia, Medellín, Colombia, E-mail: [angelgonzalezmarin1972@gmail.com](mailto:angelgonzalezmarin1972@gmail.com).

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