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ORIGINAL ARTICLE

WILEY mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Clinical mycology in Latin America and the Caribbean: A snapshot of diagnostic and therapeutic capabilities

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Summary

Despite the existence of endemic mycoses in Latin America and the Caribbean, in addition to a large population of patients at risk for invasive mycoses, the capability of medical centres to perform a proper diagnosis in mycology has not been studied in the region. Moreover, availability of antifungal drugs in the region is unknown. Here, we report the results of a survey involving 129 centres in 24 countries. Only 9% of centres would have the potential to apply for the minimum standards in mycology, as determined by the European Confederation of Medical Mycology. There is an urgent need to improve diagnostic conditions in Latin America and the Caribbean, as well as providing access to safer and more efficacious antifungal drugs.

KEYWORDS

antigen detection, diagnosis, galactomannan, mycology, survey

1 | INTRODUCTION

The Latin America and the Caribbean region are a fertile terrain for fungi. The region contains over 670 million inhabitants and a large surface area of 19 461 731 square kilometres. The rich diversity of biomes in the area provides a wide range of habitats suitable for pathogenic fungi.[1] There is a large population (over 123 million people) of rural workers,[2] exposed to fungal pathogens living in soil; there are also many patients suffering from comorbidities like malignant neoplasia, asthma, tuberculosis and HIV infection, which are at great risk for fungal infections.[1,3] Brazil, the country with the largest population in the region holds the second largest kidney and liver transplant programs, in absolute numbers, in the world: this translates to a large number of immunocompromised hosts, prone to fungal infections.[4]

Despite the significant burden of fungal infections in Latin America and the Caribbean, insufficient attention to the problem seems to occur, with lack of awareness from both society and governments.[5] Histoplasmosis and paracoccidiomycosis are examples of endemic infections with high incidence rates in the region.[6] In a similar fashion as in the developed world, opportunistic infections like aspergillosis and candidosis affect an emergent contingent of immunocompromised hosts in Latin America and the Caribbean. Here, we present the results of a survey that aimed to document the ability of local sites in Latin America and the Caribbean to diagnose fungal infections in the region.

2 | METHODS

An online survey was planned in late 2017 and kept online between February to September 2018. A 33-item questionnaire, approaching institution sizes and profiles, diagnostic procedures and tools, perceptions about fungal infection using a Likert scale, and antifungal drug access was constructed and assessed by the participants. We aimed to contact the greatest possible number of institutions in the region. Contacts were made through LIFE initiative (Leading International Fungal Education), SBI (Brazilian Society of Infectious Diseases), API (Asociación Panamericana de Infectología), SBAC (Brazilian Society of Clinical Analysis) and SBM (Brazilian Society of Microbiology). Members of such societies were invited by e-mail to participate during the first 2018 trimester. The questionnaire was built in three languages: Spanish, Portuguese and English (links for questionnaires available in Supplementary Material).

Data was scrutinised for repetitions or missing information; repeated data (more than 1 answer from the same institution) were

FIGURE 1 Geographic location of centres participating in this survey of diagnostic capabilities in Latin America and the Caribbean. Yellow marks represent institutions that responded to the questionnaire. Yellow marks with a black central star are institutions with the potential to obtain ECMM Blue status by the European Confederation of Medical Mycology (ECMM)

excluded. Incomplete or incongruent responses were solved by contacting institutions, whenever possible. Statistical analysis was made using JMP 9.0 (SAS Institute, Calgary, USA). Chi-square tests and descriptive statistics were utilised. Statistical significance was set at <0.05.

We used the definitions of the European Confederation of Medical Mycology (ECMM) to classify mycology laboratories according to their excellences in four categories: Blue, Silver, Gold and Diamond (https://www.ecmm.info/wp-content/uploads/ECMM-Excellence-Centers-Clinical-Quality-Audit.pdf). A minimum requirement for the Blue status would be identification of relevant yeasts and moulds; susceptibility testing on yeasts and moulds according to standard procedures; performance of antigen ELISA for *Aspergillus;* and cryptococcal antigen test. These criteria take into consideration not only the laboratorial ability to diagnose fungal infections, but also how these infections are managed.

3 | RESULTS

3.1 | Institutions

We received a total of 129 responses. Countries included Brazil (n = 96), Mexico (n = 9), Colombia (n = 5), Uruguay (n = 3), Guatemala (n = 3), Argentina (n = 2), Chile (n = 2), Paraguay (n = 2), Venezuela (n = 2), Barbados (n = 1), Ecuador (n = 1), Honduras (n = 1), Peru (n = 1) and French Guyana (n = 1). Figure 1 illustrates on a map the geographic distribution of centres participating in the survey. Number of hospital beds varied between 12 and 3000 (median, 203 beds). Six respondents were from laboratories or clinics not located in a hospital.

Most frequent institution profiles were public (n = 50; 38.8%), university hospitals (n = 46; 35.7%) and private hospitals (n = 18; 14.0%). Two institutions (1.6%) reported a mixed profile (public and private). Intensive care unit beds ranged 3-500 (median 15 beds). Institutions provided care for HIV/AIDS (n = 107; 82.9%), oncology (n = 86; 66.7%), haematology (n = 81; 62.8%), solid organ transplant (n = 50; 38.8%) and haematopoietic stem cell transplant (n = 43; 33.3%).

3.2 | Laboratory structure

The majority of institutions (n = 101; 78.3%) performed microbiology diagnostics in its own laboratory. Other institutions (n = 24; 22.0%) had an outsourced microbiology laboratory. Brazil had a significantly higher number of outsourced microbiology laboratories than other countries in the region (26/90 vs 1/33; $P = 0.002$). A small fraction (n = 9; 7%) of responders reported no access to any mycology diagnostic procedures, and 7 of these (77.8%) were hospitals located in Brazil.

3.3 | Perception of fungal disease

A total of 36% of responders perceived the incident of fungal diseases at their institutions as moderate (Likert 3). Perceptions varied greatly, with 16% of institutions reporting very low incidence (Likert 1) and 11% reporting very high prevalence (Likert 5). The pathogen of importance most reported was *Candida* spp. (94%), followed by *Aspergillus* spp. (56%), *Cryptococcus* spp. (67%) and *Histoplasma capsulatum* (48%).

3.4 | Microscopy, staining and fluorescence

Regarding microscopy, potassium hydroxide was used in 83/123 (68%) of institutions. India ink for cryptococcosis was ordinarily performed in 119/129 (92%) of centres. In the clinical suspicion of pneumocystosis, silver stain was available for 53/127 (42%). Fluorescence dyes were used in only 34/123 (28%). Giemsa stain was reported in 74/123 (60%) and other methods in 29/123 (24%).

3.5 | Fungal identification

Identification methods used by the institutions in this survey are summarised in Figure 2. Automated blood cultures for fungi have been used in 99/127 (78%) institutions. Most institutions reported use of classic biochemical tests, and automated systems (ie, VITEK©). A total of 55/121 institutions (45%) were able to identify moulds at the genus level only; for yeasts that occurred for 17/122 centres (14%). MALDI-ToF was in place for fungal identification in 23/114 (20%) of centres; 20/114 (18%) have access to DNA sequencing for such purpose. Brazil has significantly less institutions able to identify fungi at the species level, in comparison to other countries in the study (23/96 vs 16/33; $P = 0.008$).

3.6 | Antifungal susceptibility testing

A significant percentage (39%) of institutions did not have access to antifungal susceptibility tests. Most centres (46%) reported testing

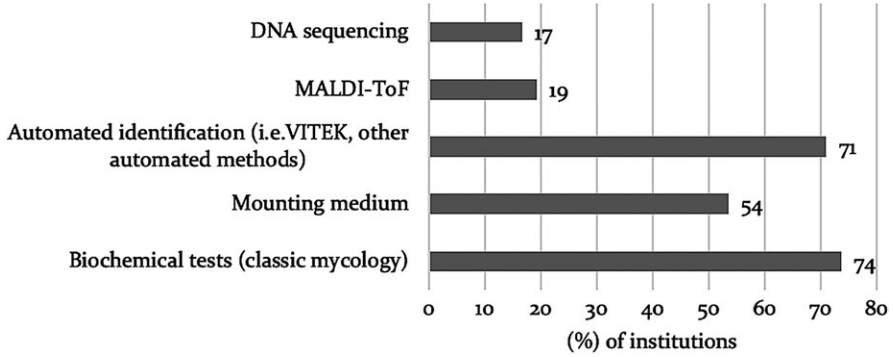

FIGURE 2 Methods for fungal identification in responders' institutions. Bars indicate percentage of institutions with availability

only for yeasts; 2% only for moulds, but not yeasts. Fifteen laboratories (12%) performed antifungal susceptibility testing for yeasts and moulds. Most institutions in the region used automated methods for susceptibility tests (50/82; 61%). E-test® was also frequently reported (35/82; 43%). Brazil had significantly less access to antifungal susceptibility tests (19% and 45%; $P = 0.011$), in comparison to other countries in Latin America and the Caribbean, respectively.

3.7 | Serology

Fungal serology was available for some of the responders: 51% (48/94 centres) informed availability of *Aspergillus* spp. serology, 54% (51/94) of *Histoplasma capsulatum* serology and 47% (44/94) for *Paracoccidioides brasiliensis* serology tests. Brazil had significantly more availability of *Paracoccidioides brasiliensis* serology test (66% vs 17%; $P < 0.001$ in comparison to other countries in Latin America and the Carabbean, respectively). Overall, 47% of institutions reported that serology tests were undertaken in an outsourced laboratory.

3.8 | Antigen testing

Cryptococcal antigen (CrAg®) detection was the most frequently available antigen test in Latin America and the Caribbean, with 75% (80/107) of institutions reporting routine use of CrAg®. *Aspergillus* galactomannan detection was performed in 48% (51/107) of centres. Other antigen tests such as *Histoplasma capsulatum* antigen detection and 1,3-Beta-D-Gllucan test were less used by the responders: 22% (24/107) and 17% (18/107), respectively.

3.9 | Molecular tests

There were scarce responders reporting availability of molecular tests, with most (59%) institutions performing outsourced testing. *Pneumocystis*- and *Candida*-targeted PCR were the most available tests (both 19/73 institutions; 26%), followed by *Aspergillus* PCR (15/73; 20%). Other PCR against different fungi were reported by 22% (16/73) of centres, and 16% (12/73) informed execution of other molecular tests.

3.10 | Therapeutic drug monitoring

Access to therapeutic drug monitoring (TDM) was limited to responders: voriconazole was the drug more frequently measured (16%), followed by itraconazole (10%) and posaconazole (4%). Most serum drug measurements were made in outsourced laboratories (79%).

3.11 | Therapy

Antifungal access for centres participating in this survey is demonstrated in Table 1. Most available drugs were fluconazole and amphotericin B deoxycholate. 5-Flucytosine (5-FC) was among the less available antifungal therapies, along with isavuconazole, echinocandins, and lipid formulations of amphotericin B.

3.12 | Overall evaluation using ECMM standards

Using ECMM criteria for laboratory Blue status, we identified only 11 institutions (9% of total) that currently have the potential to fulfil these criteria (Figure 1). Five of these centres are located in Brazil; the others are located in Mexico (n = 3), Colombia (n = 2) and Chile (n = 1).

4 | DISCUSSION

This survey is the largest and most updated snapshot of the clinical mycology scenario in Latin America and the Caribbean. We observed

TABLE 1 Access to antifungal drugs in institutions located in Latin America and the Caribbean

Antifungal drug	Overall (n = 124)	Brazil (n = 94)	Other countries (n = 30)
Fluconazole	121 (98%)	92 (98%)	29 (97%)
Itraconazole	88 (71%)	65 (69%)	23 (77%)
Voriconazole	68 (55%)	44 (47%)	24 (80%)
Posaconazole	26 (21%)	13 (14%)	13 (43%)
Isavuconazole	1 (1%)	0 (0%)	1 (3%)
Amphotericin B deoxycholate	89 (72%)	68 (72%)	21 (70%)
Liposomal amphotericin B	60 (49%)	40 (43%)	20 (67%)
Other lipid formulations of amphotericin B	52 (42%)	43 (46%)	9 (30%)
Micafungin	51 (41%)	45 (48%)	6 (20%)
Anidulafungin	39 (32%)	30 (32%)	9 (30%)
Caspofungin	37 (30%)	15 (16%)	22 (73%)
5-Flucytosine	10/55 (18%)	7/40 (18%)	3/15 (20%)

that, despite the high incidence of endemic fungal infections in the region,[6] and the existence of at-risk populations for opportunistic fungal infections,[7-9] many medical institutions are not well prepared for the challenge. In general, there is an important lack of diagnostic capabilities and therapy that impairs the adequate diagnosis and management of fungal diseases. There were few studies approaching the problem in different parts of the world. Some of them share this same problem in gathering data from institutions.[10-14]

ECMM provide an accreditation to medical centres in the subject of medical mycology. This accreditation is based on best practice recommendations published elsewhere[15] and takes into account laboratory and clinical practices. ECMM classifies institutions as Blue, Silver, Gold or Diamond. Our questionnaire could identify centres fulfilling criteria for potential ECMM Blue status, even though we did not evaluate other important clinical aspects of ECMM audits. Overall, few responders (9%) could be classified as ECMM potential Blue status, a fact that illustrates the difficulties for institutions in Latin America and the Caribbean to achieve European standards—to be noted, the most inferior classification according to ECMM.

There is a worrisome situation regarding cryptococcosis. One-quarter of the institutions surveyed reported no access to CrAg®. Considering the HIV epidemic in Latin America and the Caribbean,[16] this could be leading to significant mortality—by having a delayed or even an unreliable diagnosis, taking into account that cryptococcal meningoencephalitis is a major contributor to mortality in people living with HIV.[17,18] The situation gets even worse when we track the availability of 5-FC. 5-FC is an extremely important drug to treat cryptococcosis: it significantly reduces mortality when added to amphotericin B,[19] and it proved to be effective in an "all-oral" regimen added to fluconazole.[20] Nevertheless, less than one-fifth of responders reported access to 5-FC.

Antifungal susceptibility tests are not widely available, especially in Brazil. This could lead to inadequate treatments and unfavourable outcomes. Moreover, the lack of epidemiological data to understand the scenario of antifungal resistance in this region may be catastrophic. The widespread use of antifungals in human health, agriculture, antifouling coatings and timber preservation may cause pressure for antifungal resistance[21] and since these activities are increasing in a developing area like Latin America and the Caribbean, the next challenge could be antifungal resistance in human pathogens. Definitely, the region is not well prepared for this challenge.

Some regional disparities were evidenced: even though Brazil has the strongest economy in the region, the practice of outsourcing was more common and there is significantly less access to antifungal susceptibility tests in this country, suggesting a low level of awareness for fungal diseases. Lack of access to antifungal drugs was common: echinocandins, first-line of therapy for invasive candidosis is not available in over 50% of responders. On the other hand, Brazil concentrated most top-level institutions, with access to modern technologies like MALDI-ToF and fungal DNA sequencing, as well as antifungal TDM. Overall, TDM was reported scarcely available by responders, even though the majority of institutions have access to voriconazole TDM. The use of voriconazole particularly in paediatric patients requires TDM which can significantly improve clinical outcomes.[22]

The Ministry of Health in Brazil has a program for endemic mycoses. In this program, physicians facing with a patient with endemic mycoses have to contact a representative from the Brazilian government in order to get access by post to either amphotericin B lipid complex (ABLC) or itraconazole capsules. However, the government has not yet created a network of centres providing diagnosis for those institutions that are in need for this, which make this program partially efficient at best. Moreover, the choice for ABLC does not seem to rely on scientific grounds. Liposomal amphotericin B (L-AmB) is known for being less toxic than ABLC,[23] and L-AmB was the only drug that showed superiority over amphotericin B deoxycholate in a clinical trial, in AIDS patients with histoplasmosis.[24] As already mentioned, 5-FC is barely available in Brazil, and itraconazole solution (which is better absorbed than itraconazole capsules) is just not available in the country. Medical societies should also play their part by elaborating guidelines that clearly show the indication for different antifungal drugs, in addition to diagnostic tests in mycology.

We undertook an effort to include as many institutions as possible, however, our sample size and location of most centres in Brazil probably constitutes a certain limitation in our study. Also, we had to make this questionnaire relatively short, in order to facilitate adherence. As a consequence, we did not ask for important questions, including time to obtain a laboratory result, quality control in place, price for services, and the use of generic/brand formulations of antifungal drugs.

In summary, we demonstrated that the Latin America and the Caribbean region has a lack of diagnostic tools and availability of therapy which is a worrisome finding for a region with a high incidence of endemic and opportunistic mycoses. Efforts should be made to improve diagnostic capabilities and equalise regional disparities.

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CONFLICT OF INTEREST

Dr. Falci has received payment for lectures and/or travel reimbursements from TEVA, Pfizer, United Medical and Gilead Sciences. Dr. Pasqualotto has received research grants from Gilead, Pfizer, and MSD. He has given payed talks or participated in advisory board meetings for Gilead, Pfizer, MSD, and Astellas pharma.

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REFERENCES

1. Colombo AL, Tobon A, Restrepo A, Queiroz-Telles F, Nucci M. Epidemiology of endemic systemic fungal infections in Latin America. *Med Mycol*. 2011;49(8):785-798.
2. International Labour Organization. Working in Rural Areas in the 21st Century: Reality and Prospects of Rural Employment in Latin America and the Caribbean. In. Lima, 2016.
3. Giacomazzi J, Baethgen L, Carneiro LC, et al. The burden of serious human fungal infections in Brazil. *Mycoses*. 2016;59(3):145-150.
4. Associação Brasileira de Transplantes de Órgãos. Organ Transplantation in Brazil. In. São Paulo, 2016.
5. Tudela JLR, Denning DW. Recovery from serious fungal infections should be realisable for everyone. *Lancet Infect Dis*. 2017;17(11):1111-1113.
6. Queiroz-Telles F, Fahal AH, Falci DR, Caceres DH, Chiller T, Pasqualotto AC. Neglected endemic mycoses. *Lancet Infect Dis*. 2017;17(11):e367-e377.
7. Lewis RE, Cahyame-Zuniga L, Leventakos K, et al. Epidemiology and sites of involvement of invasive fungal infections in patients with haematological malignancies: a 20-year autopsy study. *Mycoses*. 2013;56(6):638-645.
8. Maschmeyer G, Haas A, Cornely OA. Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. *Drugs*. 2007;67(11):1567-1601.
9. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev*. 2007;20(1):133-163.
10. Chindamporn A, Chakrabarti A, Li R, et al. Survey of laboratory practices for diagnosis of fungal infection in seven Asian countries: an Asia Fungal Working Group (AFWG) initiative. *Med Mycol*. 2018;56(4):416-425.
11. Lasseter G, McNulty CA, Palmer M, Yoxall H, Kibbler C. Developing best practice for fungal specimen submission–fungal audit of general practice. *Mycoses*. 2012;55(6):476-482.
12. Morris AJ, Arthur IH, Kidd SE, et al. Mycological testing of clinical samples in Australasian pathology laboratories: wide diversity and room for improvement. *Pathology*. 2016;48(6):531-534.
13. Rosner ER, Reiss E, Warren NG, Shadomy HJ, Lipman HB. Evaluation of the status of laboratory practices and the need for continuing education in medical mycology. *Am J Clin Pathol*. 2002;118(2):278-286.
14. Schelenz S, Barnes RA, Kibbler CC, Jones BL, Denning DW. Standards of care for patients with invasive fungal infections within the United Kingdom: a national audit. *J Infect*. 2009;58(2):145-153.
15. Schelenz S, Barnes RA, Barton RC, et al. British Society for Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases. *Lancet Infect Dis*. 2015;15(4):461-474.
16. Wolff MJ, Giganti MJ, Cortes CP, et al. A decade of HAART in Latin America: long term outcomes among the first wave of HIV patients to receive combination therapy. *PLoS ONE*. 2017;12(6):e0179769.
17. Crabtree Ramirez B, Caro Vega Y, Shepherd BE, et al. Outcomes of HIV-positive patients with cryptococcal meningitis in the Americas. *Intern Soc Infect Dis*. 2017;63:57-63.
18. Lawrence DS, Boyer-Chammard T, Jarvis JN. Emerging concepts in HIV-associated cryptococcal meningitis. *Curr Opin Infect Dis*. 2019;32(1):16-23.
19. Day JN, Chau TTH, Wolbers M, et al. Combination antifungal therapy for cryptococcal meningitis. *N Engl J Med*. 2013;368(14):1291-1302.
20. Molloy SF, Kanyama C, Heyderman RS, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. *N Engl J Med*. 2018;378(11):1004-1017.
21. Fisher MC, Hawkins NJ, Sanglard D, Gurr SJ. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science (New York, NY)*. 2018;360(6390):739-742.
22. Luong ML, Al-Dabbagh M, Groll AH, et al. Utility of voriconazole therapeutic drug monitoring: a meta-analysis. *J Antimicrob Chemoter*. 2016;71(7):1786-1799.
23. Falci DR, da Rosa FB, Pasqualotto AC. Comparison of nephrotoxicity associated to different lipid formulations of amphotericin B: a real-life study. *Mycoses*. 2015;58(2):104-112.
24. Johnson PC, Wheat LJ, Cloud GA, et al. Safety and efficacy of liposomal amphotericin B compared with conventional amphotericin B for induction therapy of histoplasmosis in patients with AIDS. *Ann Intern Med*. 2002;137(2):105-109.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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