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MEDICINE AND PUBLIC HEALTH - RESEARCH PAPER

# Antifungal susceptibility and multilocus sequence typing (MLST) of *Cryptococcus neoformans* complex from HIV-associated cryptococcal meningitis patients in Manaus, Amazonas, Brazil

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## Abstract

*Cryptococcus neoformans* is primarily responsible for cases of cryptococcal meningitis in individuals with HIV/AIDS. This study evaluated the susceptibility of *C. neoformans* obtained from individuals with cryptococcal meningitis associated with HIV/AIDS in Manaus, Amazonas, Brazil, against the action of the antifungals amphotericin B, flucytosine, fluconazole, itraconazole and posaconazole and analyzed it using Multilocus Sequence Typing (MLST) in order to identify the Sequence Types (STs). We analyzed 30 isolates of *C. neoformans*, from 24 HIV/AIDS patients diagnosed with cryptococcosis from 2017 to 2019 in a reference hospital, in addition to 3 environmental isolates: 1 isolate obtained in the home of a patient and 2 isolates obtained from neighboring homes of patients. 86.6% (n = 26/30) of the clinical isolates were identified as *C. neoformans* VNI ST93, 6.6% (n = 2/30) as *C. neoformans* VNI ST5, 3.3% (n = 1/30) as *C. neoformans* VNI ST32 and 3.3% (n = 1/30) as *C. neoformans* VNB ST232. The environmental isolates were identified as *C. neoformans* VNI ST93 (n = 3/3). 96.6% (n = 29/30) isolates were sensitive to amphotericin B, though there was variation in the MIC. 60% (n = 18/30) presented a MIC above the proposed epidemiological cutoff values for one or more antifungals. All environmental isolates were sensitive to the tested antifungals. The MLST showed that there is an important relationship between *C. neoformans* VNI ST93 and individuals with HIV/AIDS, including in the environmental isolates analyzed. *C. neoformans* VNB ST232 was observed for the first time in Amazonas. Amphotericin B was proven to be the best drug, but fluconazole and posaconazole also showed relevant action.

**Keywords** Cryptococcosis · *Cryptococcus* · HIV/AIDS · Antifungals · MLST · Amazonas

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## Introduction

Cryptococcosis is a global invasive fungal infection with considerable morbidity and mortality [1], and cryptococcal meningitis is one of the opportunistic infections that most commonly affects individuals with HIV/AIDS [2]. In Latin American countries, *C. neoformans* VNI is the main agent of cryptococcosis associated with HIV/AIDS [3].

In Brazil, although the public health system provides free antiretroviral therapy (ART), the mortality rate for cryptococcal meningitis caused by *C. neoformans* still remains high [4]. According to the international standard induction regimen, the treatment of cryptococcal meningitis is performed with amphotericin B and flucytosine, followed by fluconazole [5]. However, in Latin America, flucytosine is not available and the induction of combined therapy, of amphotericin with fluconazole, is not frequent [6]. Adequate therapy associated with early diagnosis of the causative agent is essential in order to reduce deaths and sequelae of cryptococcal meningitis; however, the emergence of strains resistant to the antifungals used for the treatment of cryptococcosis is a factor that further hinders effective antifungal therapy [7].

In the state of Amazonas, northern Brazil, cases of meningitis with various etymologies have been observed since 1976 [8]. AIDS-associated cryptococcosis has been linked primarily to *C. neoformans* VNI with ST93 [9]. Alves et al. [10] reported a high mortality rate from cryptococcal meningitis in HIV/AIDS patients in Manaus, Brazil, which reflects the importance of cryptococcosis as an AIDS-defining disease and an important public health problem in the region, in addition to indicating that the home environment is a potential source of infection/reinfection by *C. neoformans* VNI. It is evident that epidemiological surveillance of the antifungal resistance of *Cryptococcus* in the Amazon region is very important in view of the high lethality of cryptococcal meningitis [7].

The present study aimed to determine the susceptibility of *Cryptococcus neoformans* isolates obtained from individuals with HIV/AIDS and diagnosed with cryptococcal meningitis in a reference hospital for AIDS cases in Manaus, Brazil, and identify the sequence types (STs) responsible for infections in patients via multilocus sequence typing (MLST).

## Methods

### Acquisition of the isolates and ethical approval

During the period from 2017 to 2019, 30 clinical isolates of *C. neoformans* were obtained from 24 patients with HIV/AIDS and diagnosed with cryptococcosis at the Fundação de Medicina Tropical Doutor Vieira Dourado (FMT-HVD). To verify the possibility of new infection or reinfection by different cryptococcosis agents, all isolates from the same patient obtained during the study period were analyzed. The isolates were maintained on Sabouraud dextrose agar at 4 °C in the Mycology Laboratory at FMT, and were given to us for the experiments that were carried out in the Mycology Laboratory of the Instituto Leônidas e Maria Deane (ILMD/FIOCRUZ).

In addition to the clinical isolates, three environmental isolates of *C. neoformans* obtained from the homes of patients and their neighbors were also used. Environmental samples of household dust, soil, bird excreta and atmospheric air were collected from 51 households (17 from patients and 34 from neighboring households) in order to verify the presence of the fungus in the homes of patients and their neighbors [10]. Of these, the three isolates of *C. neoformans* used in this study were obtained: one isolate from soil from a patient's home and two isolates from household dust from the homes of neighbors of patients.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Fundação de Medicina Tropical Doutor Vieira Dourado (protocol code CAAE No. 82715917.4.0000.0005).

### Multilocus sequence typing of *C. neoformans*

The isolates of *C. neoformans* were processed individually for the extraction of total DNA, according to the instructions of the QIAamp Tissue and Blood extraction kit (Qiagen, Hilden, Germany) with modifications in the pre-phase of mechanical maceration with glass beads.

Molecular typing using PCR–RFLP was performed with amplification of the primers URA5 (5´ATGTCCTCCCAA GCCCTCGACTCCG 3´) and SJ01 (5´ TTAAGACCTCTG AACACCGTACTC 3´), followed by enzymatic digestion with *HhaI* and *Sau961* (ThermoScientific) according to Meyer et al. [11]. *C. neoformans* isolates were submitted to MLST via amplification of CAP59, GPD1, LAC1, PLB1, SOD1, IGS1 and URA5 genes according to the conditions proposed by Meyer et al. [12].

Amplicons were purified and sequenced according to the manufacturer's instructions with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Capillary electrophoresis was performed in genetic analyzer sequencer (ABI 3130). The sequences were manually edited using Sequencher 5.3 software (Gene Codes Corporation, Ann Arbor, MI, USA) and the contigs were aligned in the MEGA v6.06 program. The sequences were analyzed in the MLST database (http://mlst.mycologylab.org) for determination of alleles and ST. To verify the genetic relationship of the isolates, a phylogenetic tree was constructed based on the

neighbor-joining (NJ) model, with bootstrap analysis using 1,000 replicates, and using related STs.

### Antifungal susceptibility test

ETEST® (bioMérieux) tapes were used with the antifungals: amphotericin B, flucytosine, fluconazole, itraconazole and posaconazole. The inoculum was prepared from *Cryptococcus* colonies grown on Sabouraud agar medium and 0.85% sodium chloride (NaCl) solution, homogenized to a 1.0 McFarland turbidity and then placed in a Petri dish containing RPMI 1640 (Himedia Laboratorios Pvt. Ltd., Mumbai), supplemented with 1.5% agar and 2% glucose, and buffered to pH 7.00 ± 0.01 with 3N-morpholino-propanesulfonic acid – MOPS (Sigma Aldrich) with a thickness of 0.4 mm ± 0.1. Epidemiological cutoff value (ECV) were evaluated according to Spinel-Ingroff et al. [13, 14]. Whonet software (version) 5.6 was used to analyze the determination of the geometric mean. To calculate the minimum inhibitory concentration 90 ($MIC_{90}$) and geometric mean, the value corresponding to 100% inhibition of fungal growth was used for amphotericin, 95% for flucytosine, and 80% for azole antifungals, according to the ETEST reading protocol. A sample of *Candida parapsilosis* ATCC 22019 was used as control.

## Results

### MLST of the clinical and environmental isolates of C. neoformans

A total of 33 isolates (30 clinical and 3 environmental) were analyzed. Among the clinical isolates, 86.6% (n = 26/30) were identified as *C. neoformans* VNI with ST93, 6.6% (n = 2/30) as *C. neoformans* VNI with ST5, 3.3% (n = 1/30) as *C. neoformans* VNI with ST32, and 3.3% (n = 1/30) as *C. neoformans* VNI with ST 232. The environmental isolates were identified as *C. neoformans* VNI with ST93 (n = 3/3) (Table 1).

Phylogenetic analysis demonstrates a relationship of great identity in the grouping of all ST93 isolates, both between environmental strains and clinical strains found in the infection acquired by patients. The *C. neoformans* VNB isolate with ST 232 and the reference sequences formed a separate cluster (Fig. 1).

### Susceptibility to antifungals

The isolates of *C. neoformans* (environmental and clinical) showed a large variation in the MIC for all the antifungal agents tested, though the greatest variation was observed for flucytosine (0.032 to > 32). Among the isolates, 40% (n = 12/30) were sensitive to all antifungals tested. 60% (n = 18/30) of the clinical isolates presented a MIC above the proposed ECVs for one or more antifungals. The isolate P33-515 presented high MICs for amphotericin, fluconazole and itraconazole; only this isolate was resistant to amphotericin B, and was sensitive only to flucytosine and posaconazole. For fluconazole susceptibility, 10% (n = 3/30) of clinical isolates demonstrated a MIC above what is established.

Isolates from the same patient, obtained at an interval of 15 days, showed different results against antifungals. While isolate P24-430/02 showed no elevated MIC for any antifungal, isolate P24-606/02 presented a MIC higher than the proposed ECVs for itraconazole and posaconazole. Isolate P39-1008 presented a MIC higher than the ECVs proposed for fluconazole and itraconazole, whereas isolate P39-1052 presented a higher MIC only for itraconazole. None of the environmental isolates presented a MIC higher than the proposed ECVs (Table 1). The geometric mean and $MIC_{90}$ can be seen in Table 2.

## Discussion

According to Ficarative, Meyer & Castañeda [3], in Latin America, the population of *C. neoformans* has less diversity in its STs than the population of *C. gattii*. In Brazil, the five molecular types VNI, VNB, VNII, VNIII and VNIV have already been identified. *C. neoformans* VNI with ST93 being the most frequent, followed by ST77, ST2, ST5 and ST23. ST93 also predominates among environmental isolates. *C. neoformans* VNII with ST40 and ST41 has been observed causing infection in humans and animals [15–17], and *C. neoformans* VNIV with ST11 and ST160 causing infection in humans [15]. *C. neoformans* VNIII is rare in Brazil with only one environmental isolate recorded [18].

Ferreira-Paim et al. [4], in southeastern Brazil, reported a higher frequency of ST93 when analyzing both clinical isolates of *C. neoformans* obtained from patients with HIV/AIDS and environmental isolates. In northern Brazil, ST93 is also the main subgenotype of VNI strains that causes cryptococcosis in individuals with HIV/AIDS [9].

Rocha et al. [9], in a study conducted from 2014 to 2016 (in the three years preceding this study), identified only ST93 in isolates of *C. neoformans* VNI, as the cause of cryptococcosis in 25 patients with HIV/AIDS, in Manaus, Brazil; while, in the present study, isolates from 24 patients were analyzed and, in addition to ST93, three other STs were also identified, ST5 and ST32 (*C. neoformans* VNI), in addition to ST232 (*C. neoformans* VNB), which via PCR–RFLP genotyping presented a molecular pattern identical to the molecular type VNII [10]. The VNB population may be underestimated due to the close

**Table 1** Description of the 33 isolates (clinical and environmental), isolate IDs, STs and MICs observed in the ETEST

| ISOLATES | | | | MIC (ETEST) | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample | Isolate ID | Molecular type | ST | AMB | 5-FC | FCZ | ITR | POS |
| Clinical | P2-782 | *CN* VNI | 93 | 0.125 | **16** | 0.75 | **0.94** | 0.016 |
| Clinical | P2-858 | *CN* VNI | 93 | 0.125 | 6 | 2 | 0.19 | 0.064 |
| Clinical | P3-793 | *CN* VNI | 93 | 0.125 | 1 | 0.125 | 0.064 | 0.032 |
| Clinical | P5-25 | *CN* VNB | 232 | 0.125 | 1.5 | 0.75 | 0.125 | 0.064 |
| Clinical | P6-137 | *CN* VNI | 93 | 0.125 | 6 | 1 | 0.094 | 0.016 |
| Clinical | P7-153–2 | *CN* VNI | 93 | 0.25 | 6 | 3 | 0.19 | 0.125 |
| Clinical | P7-193 | *CN* VNI | 93 | 0.125 | 6 | 2 | 0.19 | 0.094 |
| Clinical | P8-210 | *CN* VNI | 32 | 0.125 | 0.5 | 3 | 0.19 | 0.064 |
| Clinical | P9-330 | *CN* VNI | 93 | 0.125 | 4 | 2 | 0.125 | 0.032 |
| Clinical | P9-340 | *CN* VNI | 93 | 0.25 | 6 | 2 | 0.19 | 0.125 |
| Clinical | P12-526 | *CN* VNI | 5 | 0.25 | **> 32** | 3 | **0.38** | 0.047 |
| Clinical | P14-722 | *CN* VNI | 5 | 0.125 | 4 | 1.5 | **0.38** | 0.125 |
| Clinical | P16-03/02 | *CN* VNI | 93 | 0.19 | 4 | 1.5 | **0.38** | 0.094 |
| Clinical | P16-932 | *CN* VNI | 93 | 0.19 | 6 | 4 | **0.5** | 0.19 |
| Clinical | P20-338/2 | *CN* VNI | 93 | 0.032 | **> 32** | 1 | 0.19 | 0.047 |
| Clinical | P21-344/2 | *CN* VNI | 93 | 0.125 | **12** | 1.5 | 0.25 | 0.094 |
| Clinical | P23-428/2 | *CN* VNI | 93 | 0.125 | 0.125 | 8 | **> 32** | **1** |
| Clinical | P24-430/02 | *CN* VNI | 93 | 0.25 | 1.5 | 2 | 0.19 | 0.064 |
| Clinical | P24-606/02 | *CN* VNI | 93 | 0.125 | 0.032 | 12 | **> 32** | **2** |
| Clinical | P25-124 | *CN* VNI | 93 | 0.25 | 8 | 6 | 0.19 | 0.094 |
| Clinical | P27-354 | *CN* VNI | 93 | 0.25 | **24** | 1.5 | 0.19 | 0.094 |
| Clinical | P31-608 | *CN* VNI | 93 | 0.25 | **12** | 3 | 0.25 | 0.094 |
| Clinical | P33-515 | *CN* VNI | 93 | **> 32** | 0.032 | **> 256** | **1** | 0.094 |
| Clinical | P36-878 | *CN* VNI | 93 | 0.5 | 2 | **24** | **1** | 0.5 |
| Clinical | P35-849 | *CN* VNI | 93 | 0.5 | 0.25 | 2 | 0.19 | **0.64** |
| Clinical | P39-1008 | *CN* VNI | 93 | 0.38 | 6 | **24** | **0.75** | 0.25 |
| Clinical | P39-1052 | *CN* VNI | 93 | 0.19 | 8 | 6 | **0.38** | 0.19 |
| Clinical | P41-1072 | *CN* VNI | 93 | 0.25 | **12** | 6 | **0.38** | 0.125 |
| Clinical | P42-35 | *CN* VNI | 93 | 0.19 | 8 | 1.5 | 0.19 | 0.064 |
| Clinical | P43-107 | *CN* VNI | 93 | 0.25 | **> 32** | 0.75 | 0.125 | 0.064 |
| Environmental | HD 3b | *CN* VNI | 93 | 0.125 | 3 | 1 | 0.125 | 0.064 |
| Environmental | HS 4 | *CN* VNI | 93 | 0.19 | 8 | 2 | 0.19 | 0.064 |
| Environmental | HD 4b | *CN* VNI | 93 | 0.125 | 4 | 6 | 0.19 | 0.125 |

AMB: amphotericin-b; 5-FC: flucytosine; FCZ: fluconazole; ITR: itraconazole; POS: posaconazole; p: patient; HD: household dust; HS:household soil; MICs above the ECVs proposed by Espinel-Ingroff et al. [13, 14] are highlighted in bold

relationship between VNB and VNII, especially when only RFLP PCR is used to identify the molecular type [15].

Ferreira-Paim et al. [4] also identified ST32 in clinical isolates from patients with cryptococcosis in Minas Gerais, southeastern Brazil. ST32 is rarely found in South America, being more observed in South Africa where it appears to present worse patient survival after the first year of infection [19]. As in our study, ST93, ST5 and ST32 were related to patients with HIV/AIDS. *C. neoformans* with ST93, ST5 and ST32 has also been associated with AIDS in Asia [20, 21]. In East Asia, ST5 is predominant, being one of the most virulent STs [22], but in Brazil the isolation of this subgenotype is considered rare [4, 15].

The VNB genotype shows high virulence in Africa and in countries outside the African continent is considered rare [23]. Previously, in Brazil, VNB with ST504 and ST527 was identified in environmental isolates in the southeastern region [15], and VNB with ST 232 was identified in two clinical isolates of HIV/AIDS patients in Rio de Janeiro [16] and, more recently, in our study. According to Rhodes et al. [24], the formerly "African" VNB lineage occurs naturally in the South American environment, suggesting a migration of

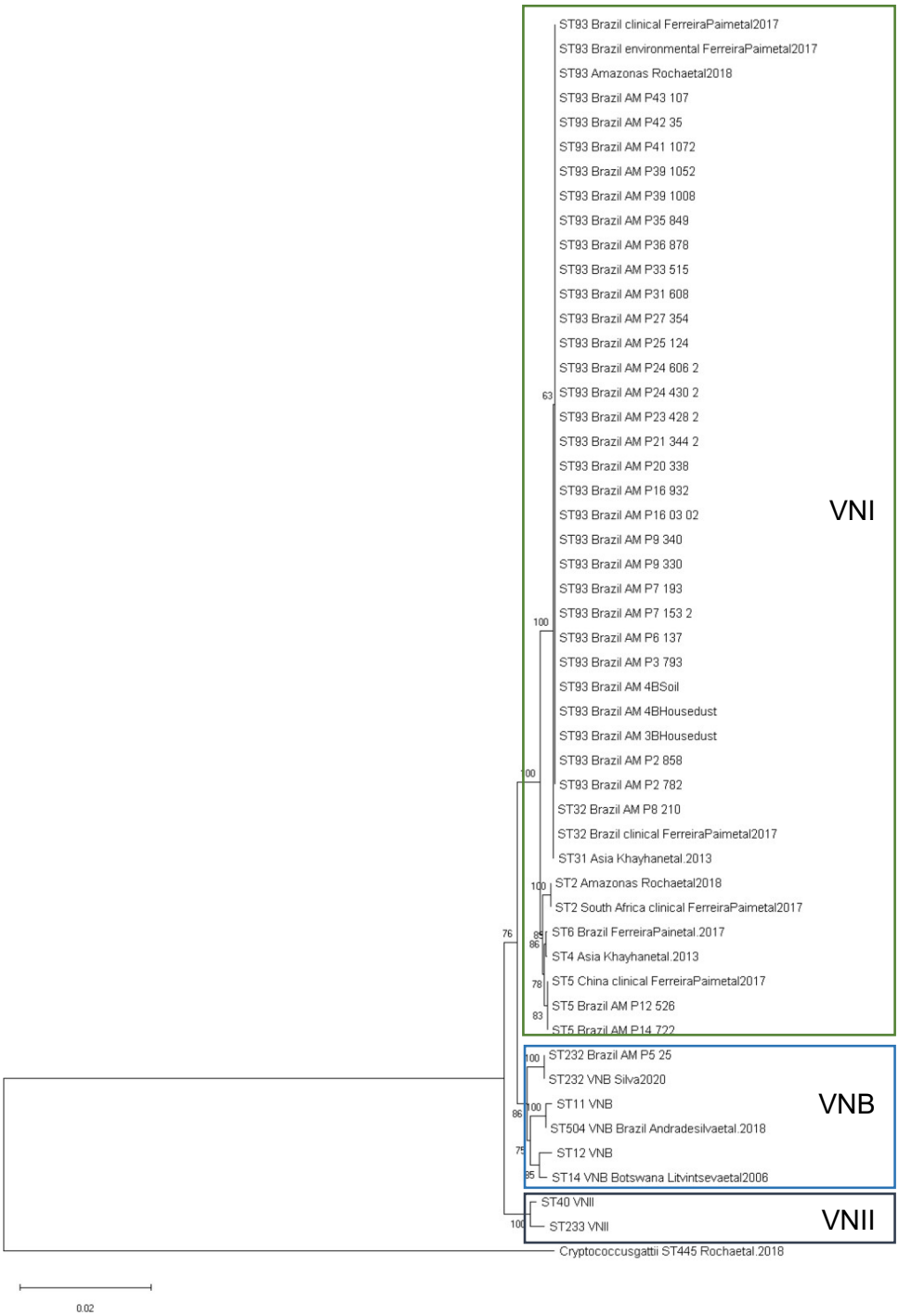


**Fig. 1** Phylogenetic tree of STs obtained in the state of Amazonas. Phylogeny reconstruction was performed by the neighbor-joining algorithm (bootstrap with 1,000 replicates) with the concatenated sequences of the CAP59, GPD1, LAC1, PLB1, SOD1, URA5 and IGS genes of the isolates obtained in the present study and sequences acquired from the MLST database

the VNB lineage between Africa and South America before the diversification of this lineage, whose nature is quite dispersive. The geographic niche of the VNB lineage could be separated when the continents split, in Pangea [25].

The three environmental isolates from patient's homes/patient's neighbors' homes are *C. neoformans* with ST93, which corroborates other environmental studies [4, 26]. Brito-Santos et al. [27] revealed an endemic pattern in domestic microenvironments in the Negro River microregion of the Brazilian Amazon and observed the presence of *C. neoformans* VNI with ST93 and also *C. neoformans* VNI with ST5.

ETEST is considered an excellent method for distinguishing yeasts that are resistant to amphotericin B [28, 29], besides being reproducible and simpler than dilution in broth [29]. There are no well-established clinical cutoff points in the revised CLSI M27-A3 and CLSI M27-S3 documents to determine the susceptibility of *C. neoformans* /*C. gattii*

**Table 2** Minimal inhibitory concentration of antifungals tested against 32 isolates (clinical and environmental) of *C. neoformans* VNI and VNB

| Antifungals | MIC range (µg/mL) | Geometric Mean (µg/mL) | $MIC_{90}$ (µg/mL) |
|---|---|---|---|
| AMB | 0.032—64 | 0.209 | 0.38 |
| FCT | 0.032—64 | 3.776 | 24 |
| FLU | 0.125—512 | 2.805 | 12 |
| ITR | 0.064—64 | 0.319 | 1 |
| POS | 0.016—2 | 0.095 | 0.25 |

isolates in tests with antifungals, so the epidemiological cutoff values (ECVs) established by Espinel-Ingroff et al. [13, 14], which proposes the adoption of species-specific values, were used. According to Lockhart et al. [30], the ECV can predict whether an isolate, for which there is not enough data to establish cutoff points, has acquired mechanisms of resistance to an antifungal agent with already known activity.

Nishikawa et al. [7], when analyzing the antifungal susceptibility of *C. neoformans* isolates from the northern region of Brazil, especially from the state of Pará, observed higher voriconazole activity among the azoles tested and lower fluconazole activity and high frequency of *C. neoformans* VNI isolates with MICs higher than the ECVs proposed for amphotericin B. Unlike the study by Nishikawa et al. [7], the present study used posaconazole and not voriconazole, and this, together with fluconazole, showed good results against the isolates analyzed. In addition, only one isolate presented MICs higher than the ECVs proposed for amphotericin B.

Rocha et al. [9] evaluated isolates of *C. neoformans* VNI with ST93 from patients with HIV/AIDS, in Manaus, Amazonas, Brazil, and via the broth microdilution technique, observed the susceptibility of all isolates to amphotericin B, fluconazole and itraconazole. This same observation is made in Silva et al. [31] and in other studies [5, 32–34]. In the present study, 60% (n = 18/30) of the clinical isolates presented MICs higher than the ECVs proposed for one or more antifungals, with variation in MICs even among ST93 isolates, and 96.6% (n = 29/30) of the clinical isolates tested were sensitive to amphotericin B.

The *C. neoformans* VNB isolate (P5-25) did not show an elevated MIC for any of the antifungals tested. Naicker et al. [35] observed low MIC values for the antifungal Fluconazole when analyzing 15 VNB isolates and no difference between the STs, including ST232.

In patients with HIV/AIDS and cryptococcal meningitis, induction therapy with amphotericin B associated with flucytosine and followed up by fluconazole for up to 10 weeks is the treatment of choice. After this period, fluconazole is used with a reduced dose according to the patient's clinical status [5]. In Amazonas state, fluconazole and amphotericin B are the drugs of choice for the treatment of patients with cryptococcosis associated with HIV/AIDS [9, 10]. Our patients were treated with these two antifungals. When evaluating the in vitro antifungal activity of Amphotericin B and Fluconazole, was observed a variation in MIC for the different isolates of *C. neoformans* VNI with ST93 and 50% (n = 10/20) of patients infected with these isolates died. The patient from whom the only strain that presented a high MIC for Amphotericin B was isolated died. This same strain also showed a high MIC for Fluconazole. The remaining patients who died were infected with isolates with MIC below the ECV for Amphotericin B and Fluconazole, but all from ST93.

The two VNI isolates of *C. neoformans* with ST5 showed high MIC for 5-FC and Itraconazole, which may demonstrate a possible resistance of this molecular type to these antifungals, but none of the patients used these antifungals and none died.

Matos et al. [36], when analyzing isolates from patients with cryptococcal meningitis in Bahia, northeastern Brazil, observed isolates resistant to amphotericin B, fluconazole and flucytosine. In our study, isolate P33-515 had elevated MICs for amphotericin B, fluconazole and itraconazole, with lower MICs for flucytosine and posaconazole. It is important to highlight that patient P33-515 used liposomal amphotericin B and died seven days after the diagnosis of cryptococcosis and initiation of treatment with this antifungal [10], which is the induction treatment in cases of cryptococcosis, administered with 2 to 4 weeks. The other isolates studied were susceptible to Amphotericin B, but P36-878 and P39-1008 showed high MIC for Fluconazole, which is used for consolidation therapy in the treatment of cryptococcosis.

In a study by Molloy et al. [37], the combination of amphotericin B plus flucytosine showed lower mortality of patients at ten weeks, but side effects were more frequent with two weeks of amphotericin B than with one week. However, as in all of Latin America, flucytosine is not available in Amazonas state. In countries where flucytosine is not available, the most frequent treatment is monotherapy with amphotericin B [6], or the induction of monotherapy with fluconazole is used; however, the rate of fungal clearance of fluconazole is lower when compared to amphotericin B, and mortality is 50 to 60% in 10 weeks, even with the use of high doses [33].

As for the environmental isolates, the MIC results were the same as those observed in most clinical isolates and susceptibility was similar to that reported in other environmental studies, though isolates resistant to the antifungals tested were not observed [38–40].

## Conclusion

*C. neoformans* VNI with ST93 is mainly involved in infections that affect individuals with HIV/AIDS in Brazil and throughout Latin America. In Amazonas state, there is a predominance of this subgenotype, but there are other STs present that affect individuals with HIV/AIDS, namely ST5 and ST32, in addition to *C. neoformans* VNB with ST232 infections having also been observed. *C. neoformans* VNB, previously considered restricted to the African continent, has been observed in other parts of the world, including Brazil. In this study, *C. neoformans* VNB with ST232 was observed for the first time in a patient with HIV/AIDS in Manaus, Brazil. ST93, the ST that is most observed in clinical isolates, was also identified in the environmental isolates analyzed, thus demonstrating the importance of the home environment for possible infection/reinfection by the fungus. In all, 40% of the isolates were sensitive to all antifungals. Only one isolate presented a MIC above the proposed ECV for AMB, which demonstrates that AMB, despite being associated with side effects, is still the most recommended and effective drug for the treatment of cryptococcal meningitis. To reduce sequelae and mortality from cryptococcal meningitis, there is a need for early diagnosis and treatment of cryptococcosis, greater adherence to treatment with HAART and other effective therapeutic options.

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## Declarations

**Conflict of interest statement** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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