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Brief communication

Molecular types of *Cryptococcus* species isolated from patients with cryptococcal meningitis in a Brazilian tertiary care hospital

Fernanda Wirth, Maria Isabel Azevedo, Luciano Z. Goldani[*]

Universidade Federal do Rio Grande do Sul, Hospital de Clínicas de Porto Alegre, Unidade de Doenças Infecciosas, Porto Alegre, RS, Brazil

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ABSTRACT

There are limited data on the molecular epidemiology of cryptococcosis in Brazil. Here, we report on the identification of the molecular pattern of the *Cryptococcus* species that caused meningitis in patients admitted in a Brazilian reference tertiary care hospital, and review the published studies addressing the molecular epidemiology of Cryptococcus in Brazil. Our study has shown the predominance of molecular type VNII in HIV-infected patients with cryptococcal meningoencephalitis. Molecular types VNII and VGII were occasionally detected in HIV-infected and non-infected patients with meningoencephalitis. In contrast, previous studies have shown that several regions exhibited a high prevalence of the VNI molecular type and sporadic cases of the VNII and VGII molecular types in patients with cryptococcosis in Brazil. Additional studies including VNII isolates will contribute to understanding the epidemiology and phylogenetic relationship of these genotype compared to the other ones. So far, no clear correlation has been established between genotypes, antifungal susceptibility for *Cryptococcus* and clinical outcome in cryptococcosis.

Cryptococcosis is a systemic mycosis primarily caused by *Cryptococcus neoformans* species complex. The *C. neoformans* complex includes the molecular types VNI, VNII, VNB, VNIII, and VNIV, and the *C. gattii* species complex, which includes the molecular types VGI, VGII, VGIII, and VGIV.[1,2] Members of the *C. neoformans* species complex are responsible for most cases of cryptococcosis associated with AIDS. Using genotyping techniques four major molecular types can be identified VNI, VNII, VNB, VNIV, in addition to the AD hybrid. VNI and VNII have a worldwide distribution.[3] In terms of serotypes, three varieties are recognized: *C. neoformans* var. *grubii*, serotype A, *C. neoformans* var. *neoformans*, serotype D, *C. neoformans* var. *gattii*, serotypes B and C, and the hybrid serotype AD.[4–6]

Meningitis is the most serious infectious due to *Cryptococcus* sp. The human immunodeficiency virus (HIV)/AIDS pandemic increased the population of immunosupressed and susceptible individuals and brought an increase *in C. neoformans* infection rates, but the increasing number of people living with any other immunodeficiency, including transplant and cancer patients, represents a growing population at risk for cryptococcosis.[7,8]

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[*] *Corresponding author.*
E-mail address: lgoldani@ufrgs.br (L.Z. Goldani).

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The recent availability of DNA fingerprinting techniques extended our knowledge about *C. neoformans* epidemiology over the past years. Naturally, the randomized amplified polymorphic DNA, PCR-fingerprinting, restriction fragment length polymorphism, karyotyping, allele sequencing, and multilocus enzyme electrophoresis helped us to answer several questions. PCR fingerprinting technique has been used as the major typing technique in the ongoing global molecular epidemiologic survey of *C. neoformans*, dividing >400 clinical and environmental isolates into eight major molecular types: VNI (var. grubii, serotype A), VNII (var. grubii, serotype A), VNIII (serotype AD), VNIV (var. neoformans, serotype D), VGI, VGII, VGIII, and VGIV (var. gattii, serotypes B and C). No correlation between serotype and molecular type has been found for *C. neoformans var. gattii*. The molecular types were recently confirmed by RFLP analysis of the orotidine monophosphate pyrophosphorylase (URA5) gene and the phospholipase (PLB1) gene.[9–13]

Currently, there are limited data on the molecular epidemiology of cryptococcosis in Brazil. Here, we report on the identification of the molecular pattern of the *Cryptococcus* species that caused meningitis in patients admitted in a reference Brazilian tertiary care hospital, and review the published studies addressing the molecular epidemiology of *Cryptococcus* in Brazil.

Cryptococcus sp. was isolated from the cerebrospinal fluid of patients with meningoencephalitis admitted at Hospital de Clinicas de Porto Alegre, 700-bed tertiary Brazilian reference hospital, from January 2014 to December 2015. Initially, *C. neoformans* and *C. gattii* were distinguished by a phenotypic method – CGB agar (cavanine glycine bromothymol blue agar). Detailed demographics, risk factors, treatment regimens and clinical outcome of the patients with meningeal cryptococcosis were evaluated from medical records. The study was approved by the local ethics committee. *Cryptococcus* complex reference strains were very kindly provided by Laboratory of Mycology (Pathogenic Fungi Collection), at Oswaldo Cruz Foundation (FIOCRUZ) – INI/FIOCRUZ in Brazil. The strains included were the following: WN = M 148 (VNI, serotype A), WM 626 (VNII, serotype A), WM 628 (VNIII, serotype AD), WM 629 (VNIV, serotype D), WM 179 (VGI, serotype B), WM 178 (VGII, serotype B), WM 161 (VGIII, serotype B) and WM 779 (VGIV, serotype C). The extraction of genomic DNA and RFLP analysis was performed according protocol using 2 primers: URA5 (5′ATGTCCTCCCAAGCCCTCGACTCCG3′) and SJ01 (5′ TTAAGACCTCTGAACACCGTACTC3′). RFLP patterns were assigned visually by comparing them to the patterns obtained from the standard-type strains (VNI-VNIV and VGI-VGIV).[14,15]

Fifteen patients with cryptococcal meningoencephalitis were evaluated in the study (Table 1). The gene URA-5 RFLP showed that nine patients had VNII, serotype A, two patients VNI serotype A, and two had VGII, serotype B isolate from their CSF samples. Two patients presented a positive CSF cryptococcal polysaccharide antigen, and *Cryptococcus* could not be isolated from the CSF samples. As expected, the VNII, serotype A strains were isolated from patients with underlying immunossupressive disease. HIV positive was the most common condition, followed by kidney and liver transplant. Meningoencephalitis by *C. gattii* was diagnosed in two patients. One of them was immunocompetent and the other presented an immunosuppressive condition (HIV). Clinical isolates were highly susceptible to amphotericin B and fluconazole by microdilution method (data not shown). In Brazil, studies of the *in vitro* susceptibility of *Cryptococcus* spp. have shown that most isolates are susceptible to polyene antifungals, 5-flucytosine, and azoles.[16]

Although we analyzed a small number of isolates, our study has shown the predominance of VNII *Cryptococcus* genotype

Table 1 – *Cryptococcus* species complex and genotype identification in the CSF samples from patients with cryptococcal meningitis.

	Age/Sex	Species complex	Molecular type	Underlying disease	Treatment	Outcome
1	54/M	*C. neoformans*	VNII, Serotype A	Kidney transplant	Liposomal AmB + FCZ	Death
2	6/M	*C. neoformans*	VNII, Serotype A	Liver transplant	AmB + 5-FC	Alive
3	45/M	Not available	Not available	HIV+	AmB + FCZ	Death
4	27/M	*C. neoformans*	VNII, Serotype B	HIV+	AmB + FCZ	Alive
5	65/M	*C. gattii*	VGII, Serotype B	Immunocompetent	AmB + FCZ (VCZ)	Death
6	33/F	*C. neoformans*	VNII, Serotype A	Kidney transplant	AmB + FCZ	Alive
7	42/M	*C. neoformans*	VNII, Serotype A	HIV+	AmB + FCZ	Alive
8	55/M	*C. neoformans*	VNII, Serotype A	HIV+	AmB + FCZ	Death
9	62/M	*C. neoformans*	VNII, Serotype A	Kidney transplant	AmB + FCZ	Alive
10	25/F	*C. neoformans*	VNII, Serotype A	HIV+	AmB + FCZ	Alive
11	38/M	*C. neoformans*	VNI, Serotype A	HIV+	Lipid complex AmB + FCZ	Alive
12	44/F	*C. neoformans*	VNII, Serotype A	HIV+	AmB + FCZ	Alive
13	37/F	*C. neoformans*	VNI, Serotype A	HIV+	AmB + FCZ	Death
14	41/M	*C. gattii*	VGII, Serotype B	HIV+	AmB + FCZ	Death
15	43/F	Not available	Not available	HIV+	AmB + FCZ	Death

M, male; F, female; AmB, amphotericin B deoxycholate; FLZ, fluconazole; VCZ, voriconazole.

Table 2 – Studies addressing the prevalence of *Cryptococcus* molecular types in cryptococcosis in Brazil.

Reference	Location – state	Predominant molecular type	Other molecular types
Wirth et al. (present case)	Rio Grande do Sul	VNII	VNI, VGII
Nunes et al.[18]	Mato Grosso do Sul	VNI	VNI, VGII
Andrade-Silva et al.[19]	Minas Gerais	VNI	
Aguiar et al.[20]	Minas Gerais	VNI	VNI, VGI
Ferreira-Paim et al.[21]	Minas Gerais	VNI	
Figueiredo et al.[22]	São Paulo	VNII	VGII
Favalessa et al.[23]	Mato Grosso	VNI	VGII
Freire et al.[24]	Amazonas	VNI	VNII, VGII
Matos et al.[25]	Bahia	VNI	VGII
da Silva et al.[26]	Amazonas	VNI	VGII
Martins et al.[27]	Piauí	VNI	VGII
Mora et al.[28]	Minas Gerais	VNI	VGII
Souza et al.[29]	Goias	VNI	VGII
Triles et al.[30]	RS, SP, MS, MG, BA PI, PE, RR, AM	VNI, VGII	VNII, VNIII, VNIV, VGI, VGIII
Matsumoto et al.[31]	São Paulo	VNI	VNII
Igreja et al.[32]	Rio de Janeiro	VNI, VNII	VGI, VGII
Casali et al.[33]	Rio Grande do Sul	VNI	VGIII

RS, Rio Grande do Sul; SP, São Paulo; MS, Mato Grosso do Sul; MG, Minas Gerais; BA, Bahia; PI, Piauí; PE, Pernambuco; RR, Roraima; AM, Amazonas.

in patients with meningoencephalitis. Previous studies have shown that the VNI molecular pattern is the most common pattern worldwide, whereas VNII was observed in 1%–16% of cases reported in South America, Africa, and Oceania.[17,2] *C. gattii* VGII was also observed in South America, North America, and Oceania. Table 2 describes the studies evaluating the *Cryptococcus* genotypes circulating in Brazil.[18–33] Several regions exhibited a high prevalence of the VNI molecular type and sporadic cases of the VNII and VGII molecular types in Brazil. The predominance of the VNI molecular type has been described in the States of São Paulo, Minas Gerais, Mato Grosso, Amazonas, PIauí, Rio Grande do Sul and Rio de Janeiro in Brazil.

Studies of molecular types of *Cryptococcus* contribute to the evaluation of the epidemiology, clinical manifestations, interventions and therapeutic approach for cryptococcosis. The presence of molecular type VNII was reported in all five continents, but little is known about its pathobiology, ecology, and antifungal susceptibility. Therefore, tools that are able to identify it are important to better understand how it is distributed in the environment, how it interacts with the host, and finally if it needs a different and more specific treatment to improve the patient outcome. Additional studies of VNII isolates will contribute to understanding the epidemiology and phylogenetic relationship of this genotype compared to the other ones, revealing if it represents a separate species in the *C. neoformans* species complex.

Conflicts of interest

The authors declare no conflicts of interest.

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