Variedades de *Cryptococcus neoformans* isoladas de pacientes com AIDS em hospital da cidade de São Paulo durante 1996-1999

Cryptococcus neoformans varieties isolated from patients with aids referred to a hospital of São Paulo city during 1996-1999

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RESUMO

Cryptococcus neoformans é uma levedura capsulada que apresenta tropismo pelo sistema nervoso central causando meningoencefalite. É a micose mais freqüente em pacientes com AIDS, e é responsável pela alta morbidade e mortalidade. Há duas variedades: var. *neoformans* e var. *gattii*. *C. neoformans* var. *neoformans* está distribuído mundialmente e é comumente encontrado em fezes de aves, principalmente de pombo. *C. neoformans* var. *gattii* está geograficamente limitado a regiões tropicais e subtropicais e está associado a algumas espécies de árvores, principalmente, *Eucalyptus* sp. A proposta desse estudo foi de avaliar a prevalência das variedades de *Cryptococcus neoformans* isoladas de 452 amostras de líquido cefalorraquiano (LCR) provenientes de 183 pacientes com AIDS, internados no Instituto de Infectologia Emílio Ribas – São Paulo-SP, de 1996 a 1999, utilizando meio canavanina-glicina-azul de bromotimol (CGB). Do total 452 amostras, em 446 (98,7%) foram detectados *C. neoformans* var. *neoformans* e *C. neoformans* var. *gattii* em 6 (1,3 %) amostras.
Palavras-chave. *Cryptococcus neoformans*, variedades de *Cryptococcus neoformans*, Líquido Cefalorraquiano, AIDS.

ABSTRACT

Cryptococcus neoformans is an encapsulated yeast that presents tropism for-central nervous system**,** and causes meningoencephalitis. Cryptococcosis is the most frequent mycosis in patients with AIDS, and it is the cause of high morbidity and mortality. *C. neoformans* presents two varieties var. *neoformans* and var. *gattii*. *Cryptococcus neoformans* var. *neoformans* has been world-wide isolated from avian feces, especially pigeon excreta. *C. neoformans* var. *gattii* is geographically restricted to tropical and subtropical regions, and it is associated with some trees species, mainly *Eucalyptus* sp. The proposal of this study was to evaluate the prevalence of *Cryptococcus neoformans* varieties isolated from 452 cerebrospinal fluid (CSF) samples from 183 patients with AIDS referred to the Instituto de Infectologia Emilio Ribas - Sao Paulo-SP, from 1996 to 1999. CSF samples were cultured on canavanine-glycine-bromothymol blue medium. Of 452 samples, *C. neoformans* var. *neoformans* was isolated in 446 (98.7%)**,** and *C. neoformans* var. *gattii*. in 6 (1.3%) samples.
Key words. *Cryptococcus neoformans*, cerebrospinal fluid, varieties *Cryptococcus neoformans*, AIDS.

INTRODUCTION

Cryptococcus neoformans is an encapsulated yeast that presents tropism for the central nervous system and causes meningoencephalitis[1]. Two varieties and five serotypes of *Cryptococcus neoformans* are recognized: var. *neoformans* (serotypes A, D, AD) and var. *gattii* (serotypes B e C)[2,3]. The two varieties of *C. neoformans* were differentiating by using the canavanine-glycine-bromthymol blue agar (CGB) medium[4] and the serotypes are characterize by slide agglutination tests[5,6].

In 1999, a third variety, *C. neoformans* var. *grubii*, was proposed for *C. neoformans* var. *neoformans* serotype A[7].

Cryptococcus neoformans var. *neoformans* has been isolated worldwide from avian droppings, especially pigeon excreta and soil enriched with this avian excreta[8,9,10,11]. The *C. neoformans* var. *gattii* is geographically restricted to mainly tropical and subtropical regions. The first isolation of this variety (*gattii)* was made in Australia were the investigators established its specific ecological association with *Eucalyptus camaldulensis*[12]. In this country, the molecular studies indicated that exist epidemiological association between mammalian disease and exposure[13]. To date the var. *gattii* has been isolated from native trees in the Brazil (*Moquilea tomentosa, Cassia grandis, Guettarda acreana)* and in Colombia **(***Terminala catappa*)[14,15,16,17].

In Brazil, 4.7% of acquired immunedeficiency syndrome (AIDS) related infections are caused by *C. neoformans* var. *neoformans*[18]. Since then the HAART, after toxoplasmosis, cryptococcosis is the most prevalent neurological disease in AIDS patient and a frequent AIDS-defining condition[19,20].

C. neoformans var. *gattii* rarely is the agent of cryptococcosis in AIDS patient, even in areas where the infection by this variety occurs endemically, is associated with immunosuppresed patient[1,9,21]. In the city of São Paulo both *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii* were presents in urban environment at sites where large numbers of people normally gather[22].

Some studies carried through in the states of Rio de Janeiro and São Paulo had shown that the majority of the patients were infected by the *C. neoformans* var. *neoformans* (serotype A and D) and, a minority was infected by *C. neoformans* var. *gattii* (serotype B)[21,23-30].

The purpose of this study was to verify the prevalence of *C. neoformans* varieties in AIDS patients during a period of time in an Infectology Hospital in the city of São Paulo and knowledge more about the epidemiology of this pathogen in our city.

MATERIALS AND METHODS

We retrospectively analyzed 452 *C. neoformans* isolated of cerebrospinal fluid (CSF) samples from 183 patients with AIDS admitted at the Instituto de Infectologia Emilio Ribas, in São Paulo city, during 1996 to 1999.

The laboratory diagnosis of cryptococcal infection was based on positive India ink preparation and/or positive culture of CSF in Sabouraud dextrose agar.

The identification of the colonies of yeasts was based on cellular micro morphology and by API 20C AUX (BioMerieux, France), a commercial kit.

The identification was confirmed by standard physiological and biochemical methods that included thermo tolerance at 37ºC, urease and phenoloxidase activity and assimilation tests[31].

The varieties of *C. neoformans* were determined by using the canavanine-glycine-bromothymol blue (CGB) medium. The growth and the change of color of the medium identified the variety *gattii*[2,4-31].

RESULTS

The results shown that 446 CSF samples (98.7%) from 177 AIDS patients were *C. neoformans* var. *neoformans* and 6 CSF samples (1.3%) were *C. neoformans* var. *gattii*, during 1996 to 1999, at the Instituto de Infectologia Emílio Ribas, in São Paulo city.

DISCUSSION

Prior to the AIDS epidemic, cryptococcosis occurred sporadically throughout the world[2]. Despite the incidence of cryptococcal infections has increased dramatically as a result of the AIDS epidemic, other conditions have been associated with an increased risk for *C. neoformans* infections like lymphoproliferative disorders, corticosteroid therapy and organ transplantation[8].

Pigeon excreta are the saprophytic source most commonly associated with *C. neoformans* mainly var. *neoformans* so the high prevalence of pigeons in urban area favor the exposure of humans to this variety[8].

C. neoformans var. *neoformans* is the variety overwhelmingly recovered from patients with AIDS in contrast with *C. neoformans* var. *gattii* infections that are rare in these patients[8,21].

Some studies indicated that the majority of infections in AIDS patients with cryptococcal meningoencephalitis are caused by *C. neoformans* var. *neoformans* and serotype A[9,23-30,32]. Despite the serotypes of *C. neoformans* hasn't been determined; this study is in agreement with others in which the variety *neoformans* was the more prevalent than var. *gattii* in AIDS patients.

CONCLUSION

The variety *neoformans* was the most prevalent in CSF samples from AIDS patients in Instituto de Infectologia Emílio Ribas located in São Paulo city, during 1996 to 1999.

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