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Original article

Isolation and characterization of Cryptococcus neoformans from environmental sources in Ethiopia

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Abstact

Background: Cryptococcus neoformans, the most important cause of fungal meningitis in immunocompromised patients worlwide has two varieties which differ in their geographical distribution and natural habitat.

Objective: To isolate and characterize C. neoformans from environmental sources in and around Addis Ababa.

Method: A total of 592 specimens collected from droppings of pigeons and chickens, and from material associated with eucalyptus trees were examined for the presence of C. neoformans. This study was carried out during September 1998 to July 1999.

Results: Out of the 592 specimens eleven C. neoformans were isolated from the pigeon droppings and material associated with eucalyptus trees. No C. neoformans was discovered from chicken droppings. All the isolates were identified as C. neoformans var. neoformans. C. neoformans var. gattii has not been isolated from any of the samples examined.

Conclusion: The fact that cultural detection of C. neoformans var. gattii was impossible in this study does not, however, prove the absence of this variety in these environmental sources in Ethiopia. Future studies that will include a wider geographical area in Ethiopia, and also investigate the possible association of exposure to environmental sources and risk of acquiring disease are recommended. [Ethiop. J. Health Dev. 2001;15(1):45-49]

Introduction

Cryptococcus neoformans (teleomorph, Filobasidiella neoformans) is an encapsulated round or oval yeast. It is subdivided into two varieties and four major serotypes, based on capsular epitopes: C. neoformans var. neoformans (serotypes A and D) and C. neoformans var. gattii (serotypes B and C) (1). A distinctive difference in the geographic distribution of the two varieties has been reported (2). C. neoformans var neoformans occurs worldwide while var gattii is restricted to tropical and subtropical regions (3,4).

In nature the two varieties reside in separate environmental niches, which have been identified as potential sources of pathogenic strains (5). C.neoformans var neoformans has been isolated from a variety of avian species in addition to pigeons, including chickens, parrots, sparrows and sites contaminated by pigeon excrement and at a lesser concentration in soil (6,7). C. neoformans var gattii has been isolated from Eucalyptus camaldulensis (3,4), and from Eucalyptus tereticoris (8,9). Eucalyptus trees particularly E. camaludulensis and E. globulus originated from Australia and are extensively grown in many countries (3), including Ethiopia , particularly in and around towns and cities of the highland regions. In Ethiopia E. globulus is one of the earliest introduced species, and E. terticoris and E. camaludulensis are commonly and widely planted species (10).

The disease caused by variety neoformans occurs throughout the world, while cryptococcosis caused by variety gattii is found mainly in tropical and subtropical areas (11). Cryptococcus has emerged as the fourth most common lethal infection among AIDS patients (12,13). In these patients meningoencephalitis is the most common presentation. The first case of cryptococcal meningitis in Ethiopia was described in 1992 (14). In another related study among 305 serpostive Ethiopian patients 28 developed cryptococcal meningitis (15).

The clinical isolates from AIDS patients have been identified predominantly as C. neoformans var. neoformans (16). Similar results have been obtained in Ethiopian HIV-positive patients, where all the isolates from patients were identified as serotype A (17). Isolation of strains of the variety gattii from patients with AIDS is rarely reported (18,19,20,21). It has been observed that the clinical course was prolonged, and morbidity, mortality, and severe neurological sequelae were more frequent among patients infected with C. neoformans var. gattii (22).

In Ethiopia, it is common to have chicken and pigeon droppings, and additionally eucalyptus trees around the living areas, and the possible exposure to infection is wide.

In this paper we report the first environmental isolation of Cryptococcus neoformans from different natural sources in and around Addis Ababa.

Methods

A total of 592 samples, 200(flower), 55(wet leaf), 77 (dry leaf) from Eucalyptus trees; 100 (dry), 92 (fresh) droppings of pigeon; and 20 (dry), 48 (fresh) chicken droppings were collected between September 1998 to July 1999. All the samples were from different sites in Addis Ababa and its surroundings.

The samples from eucalyptus trees were transported to the laboratory in clean dry paper envelopes. The samples were mixed with 25 ml sterile distilled water in 250 ml Erlenmeyer flasks, which were then shaken vigorously for one hour, and the contents allowed to settle for 15 min (23). From the supernatant 0.1 ml aliquot was aseptically aspirated and plated on to plates containing Guizota abyssinica creatinine agar (Bird seed agar) (24) This medium is differential for this yeast as it gives pigmented colonies. The medium was supplemented with chloramphenicol to suppress bacterial growth. The cultures were incubated at 370C and were observed daily for the development of brown yeast-like colonies.

Pigeon droppings were obtained from underneath buildings where pigeons are common. Chicken droppings (fresh and dry) were collected in sterile tubes from market places in Addis Ababa. The samples were then suspended in sterile water and shaken. The suspension is allowed to settle and aliquotes were plated on bird seed agar (BSA) and incubated at 370C.

The plates were then observed for appearance of brown colonies. All suspected colonies were subcultured on Sabouraud glucose agar and india ink preparations were made. The isolates were further identified on the basis of a positive India ink test, urease production, growth at 370C, and by carbohydrate assimilation and fermentation tests by API 20C (bioMerieux, Hazelwood, Mo.) testing (25).

The positive isolates which were confirmed to be C. neoformans, were further tested on canavanineglycine-bromthymol blue (CGB) agar for the differentiation of C.neoformans var.neoformans (serotypes A/D) and Cryptococcus neoformans var. gattii (serotypes B/C) (1).

Reference strains C. neoformans CBS 5757 and C. neoformans CBS 5756 obtained from Karolinska Institute and Hospital, Department of Clinical Microbiology, Sweden, were used as control throughout the study.

Results

Of the total 592 samples, brown-like colonies were detected on Bird seed agar on eleven cultures. These were found to be India Ink positive, urease postive and grew at 370C and gave characteristic carbohydrate assimilation profile, showing the reliability of the bird seed agar for isolation of C.neoformans. Samples of pigeon droppings yielded 5% (9/192) positivity, and samples related to eucalyptus trees yielded 0.6% (2/332) positivity (Table 1).

All the isolates identified as C. neoformans gave negative test on canavanine-glycine-brothymol blue (CGB) agar, indicating that the isolates are Cryptococcus neoformans var. neoformans (serotype A/D). From the total sample, eleven (2%) Cryptococcus neoformans var. neoformans were obtained. C. neoformans var. gattii has not been isolated from any of the samples investigated.

Discussion

The isolation of C. neoformans in nature took place for the first time from peach juice in 1895 and then from milk in 1901 (26). Then Emmons isolated the organism from soil and then from pigeon excreta in 1950s (27). Since then the two varieties have been isolated from different environmental sources.

This is the first isolation of Cryptococcus neoformans from environmental sources in Ethiopia. The percentage of positivity is well within the range reported in the literature: 0 to 24.7% (2,18,28). Nine out of the total isolates were isolated from pigeon droppings. Even though birds do not get infected because of their relative high body temperature, avian droppings are considered an optimal natural substratum for C. neoformans due to their high content of low molecular nitrogenous compounds such as creatinine (2,25).

We were unable to isolate the variety gattii in this study. This is in accordance with the preliminary work done from HIV positive patients in Ethiopia that no C. neoformnas var. gattii has been isolated (17). The presence of var. gattii was expected, since the natural habitat of this variety has been identified as eucalyptus trees which are found abundantly in Addis Ababa and its surroundings. Only five isolates of C. neoformans var. gattii were obtained from 696 specimens collected from different types of eucalyptus trees in India (6). Therefore, further more extensive studies are needed to confirm the presence or absence of variety gattii in Ethiopia.

Since C. neoformans is ubiquitous in the environment, exposure to dry inhalable yeasts from this reservoirs is expected. The small dry basidiospores would make effective infectious particles, and they have been suggested to be a source of infection (29). Even though there is a lack of direct evidence that suggests that exposure to these environmental sources is associated with an increased risk of acquiring disease there are some evidences that show that these environmental sources might be potential sources of pathogenic strains. The need of exposure to E. camaludulensis trees in order to acquire an infection by the variant gattii has been suggested as a plausible explanation for the high incidence of infections caused by this fungus in Australian aborigines and low worldwide incidence of this serotype in AIDS patients (3). Recently it has been shown that pigeon droppings contained a genetically heterogenous population of C. neoformans serotypes A and D in which some isolates are similar to infection-causing organisms demonstrating that pigeon droppings can be identified as potential source of pathogenic strains of C. neoformans (5). In previous studies genetically related isolates were found among clinical and environmental strains, suggesting that pathogenic strains of C. neoformans can be found in the environments of patients at risk for cryptococcosis (30,31). Since C. neoformans is an important pathogen in immunocompromised patients the elucidation of the chain of infection in very important.

Future studies are needed to investigate the geographic distribution of this yeast in Ethiopia, the prevalence of disease caused by this fungus, and possible direct association of disease with exposure to these natural habitats of C. neoformans.

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Tables

Table 1: ***C. neoformans* isolated from different environmental sources in and around Addis Ababa**

	Samples studied		
		Positive	
	Number	No.	%
Pigeon droppings	192	9	5
Chickens droppings	68	0	-
Eucalyptus trees	332	2	0.6
Total	592	11*	2

*All *C. neoformans var neoformans*

Figures

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