Ecological niche of *Cryptococcus neoformans* species complex from Betul city of Madhya Pradesh

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Abstract

Globally the risk of outbreaks has been increasing with the expansion of environmental *Cryptococcus neoformans* and *Cryptococcus gattii* pathogens. In this prospective study we analyzed the isolation of *C. neoformans* - *C. gattii* strains from a total of 500 tree samples and *C. neoformans* from 194 pigeon samples collected from different sites of Betul and Bhopal city of Madhya Pradesh (India). Selective isolation of *C. neoformans* sp. complex was done by swabbing and Direct Plating Method. As per the data, out of total 500 tree samples 30 were found positive for *Cryptococcus neoformans* and 36 samples positive for *C. gattii*. Highest cfu was obtained from *Tamarindus indica* ($19x10^4$). Total 35 pigeon samples were found positive for *C. neoformans* and the highest frequency was observed from the pigeon sample collected from Bablu Talab Kothin Bazar (12.08%), Betul city of Madhya Pradesh (India). This study suggested the living tree trunk hollows and pigeon excreta as a possible ecologic niche for *C. neoformans* species complex, hence it gains more attention in the environmental occurrence and role in cryptococcosis.

Keywords: *C. neoformans* species complex, living tree trunk hollows, pigeon excreta

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Introduction

Cryptococcosis is caused by both the varieties of *C.neoformans* species complex, *i.e., Cryptococcus neoformans* and *Cryptococcus gattii* that affects lungs and central nervous system predominantly and is the commonest fungal meningitis (Meyer *et al.*, 2009).

Over the past 2 decades, the case of deadly disease has increased worldwide dramatically in the number of immunocompromised individuals with HIV infection, cancer

patients receiving chemotherapy (Bovers *et al*., 2008), organ transplant recipient (Saha, *et al.*, 2007; Warkentien & Crum-Cianflone, 2010), steroid administration, sarcoidosis, diabetes or inherited immune system defects, haematological malignancies (Bassetti *et al*., 2010).

Interestingly in the concept of niche as well as worldwide dissemination state of *C. neoformans* and *C. gattii* were studied by many investigators. Casadevall & Perfect 1998; Granados & Castañeda (2005), emphasized pigeon guano as a realized niche of *C. neoformans* ince, in contrast realized niche for *C. gattii* was thought to be more restricted, which was believed to be sedentary trees (Nielsen *et al*., 2007). Yarwood *et al*., (2010), suggested that *Cryptococcus* sp. found in soil and vegetal materials may contribute to the decomposition of organic material.

Cryptococcosis distributed worldwide in association with a range of plant species (Chen *et al*., 2000; Pfeiffer and Ellis, 1992; Sorrell *et al*., 1996) and the very first environmental isolation of *C. gattii* was reported by Ellis and Pfeifer (1990) in Australia from bark, leaves, plant debris, and wood, of *Eucalyptus* trees.

In India the prevalence and distribution of *C. neoformans* was predominantly reported with droppings of pigeon (Gokulshankar *et al*., 2004; Sethi *et al.*, 1966; Gugnani *et al*., 1972), munia bird (Pal, 1989), canavies (Pal, 1995), decaying wood of *Syzygium cumini* (Randhawa *et al*., 2003; Nawange *et al.*, 2006), *Mangifera indica* (Nawange *et al*., 2006; Grover *et al*., 2007), as well as soil contaminated with pigeon excreta (Dhindsa *et al*., 1994; Nawange *et al*., 2000).

Material and methods

A) Sampling

In the present investigation, 500 environmental samples were collected from Betul and Bhopal city of Madhya Pradesh. An environmental sample includes living tree trunk hollows, and pigeon excreta from different localities inhabited by men and animals. Samples from living tree trunk hollows were collected with the help of homemade cotton swabs and pigeon excreta samples were aseptically collected using long spatulas, transferred to clean sterilized plastic bags, and properly labeled according to site and date. Samples were taken to the laboratory and were used immediately or in case of delay, samples were stored at room temperature and processed within 48 hours.

B) Isolation and sample processing

Selective isolation of *C. neoformans* sp. complex was done by swabbing and Direct Plating Method using Staib's Guizotia abyssinica Creatinine agar (GACA- Staib's) medium (50 g of pulverized Niger seeds, 1 gm dextrose, 20 gm agar, 1 gm KH2PO4, 1 gm creatinine, and pH (5.5). The medium was cooled to approximately 50ºC to which streptomycin sulfate (40 µg / ml), penicillin (20 µg / ml) and biphenyl (1 gm biphenyl in 10 ml absolute alcohol per 1000 ml of the culture medium) were added and plates were prepared.

In-house swabbing technique was used for the isolation of cryptococcal strains form includes living tree trunk hollows described by Randhawa *et al.*, (2003). In which homemade

swabs were immersed in 10 ml of sterilized physiological saline and decayed wood was collected from each trunk hollow. The sample suspension of sampled swabs was made, vortexed for 2min and allowed to sediment for 30 min, followed by inoculation of duplicate plates of NSA with 100µl aliquots of the supernatant. After 3–4 days of incubation at 28°C, the number of chocolate brown yeast-like colonies compatible with the *C. neoformans* species complex appearing on NSA plates were counted visually.

In the direct plating technique, approximately 5 g of each pigeon excreta sample was suspended in 45 ml of sterilized distilled water containing 20 mg/ml penicillin and 40 mg/ml streptomycin. The suspension was shaken vigorously for a few minutes and then was allowed to settle at 37 °C for 1 hour. Serial dilution i.e. 1:10, 1:100, 1:1000 of the suspension was prepared and 0.1 ml of each dilution was plated on GACA- Staib's medium. After 3–4 days of incubation at 28° C, the number of chocolate brown yeast-like colonies compatible with the *C. neoformans* species complex appearing on GACA- Staib's medium plates were counted visually and were subjected to biochemical, physiological and morphological identification tests in order to identify *Cryptococcus* species (Kwon-Chung and Bennet 1992; De Hoog *et al.,* (2000).

Result and discussion

The present study was carried out with the aim to understand the source and occurrence of *Cryptococcus neoformans* species complex in the Betul and Bhopal city of Central India. Isolation of both the species of *Cryptococcus neoformans* species complex was carried out from different environmental sources.

In the current investigation a total of 500 tree samples belonged to positive 13 different tree species were collected from different localities of Betul city of Central India. These thirteen living tree species with maximum isolation frequency includes *Azadirachta indica* ($15x10^3$), *A. nilotica*, ($19x10^3$), *Butea monosperma* ($12x10^3$), *D. calestachys* ($2x10^4$), *Delonix regia* (7x103), *Ficus benghalensis* ($31x10^4$), *Ficus glomerata* ($2x10^4$), *Ficus religiosa* ($16x10^3$) *M. iongiola* ($2x10^4$), *Mangifera indica* ($18x10^3$), *Pongamia pinnata* ($14x10^3$), *Pithecellobium dulce* (2x103), *P. avium* ($2x10^3$), *P. dactylifera* ($2x10^3$), *Syzygium cumini* ($3x10^4$), *Tamarindus indica* ($19x10^4$), *Zizipus mauritiana* ($18x10^4$). As per the data, 30 samples were found positive for *Cryptococcus neoformans* and 36 samples positive for *C. gattii.*

Occurrence and distribution of *Cryptococcus neoformans* was studied and found positive in 35 pigeon samples out of total 194 pigeon samples collected from different sites of Betul and Bhopal city of Madhya Pradesh (India). Highest frequency was observed from the pigeon sample collected from Bablu Talab Kothin Bazar (12.08%), followed by Denikmut office Betul (8.91%) and 8.91% in Mehamud bhai Kidwain Ward Betul.

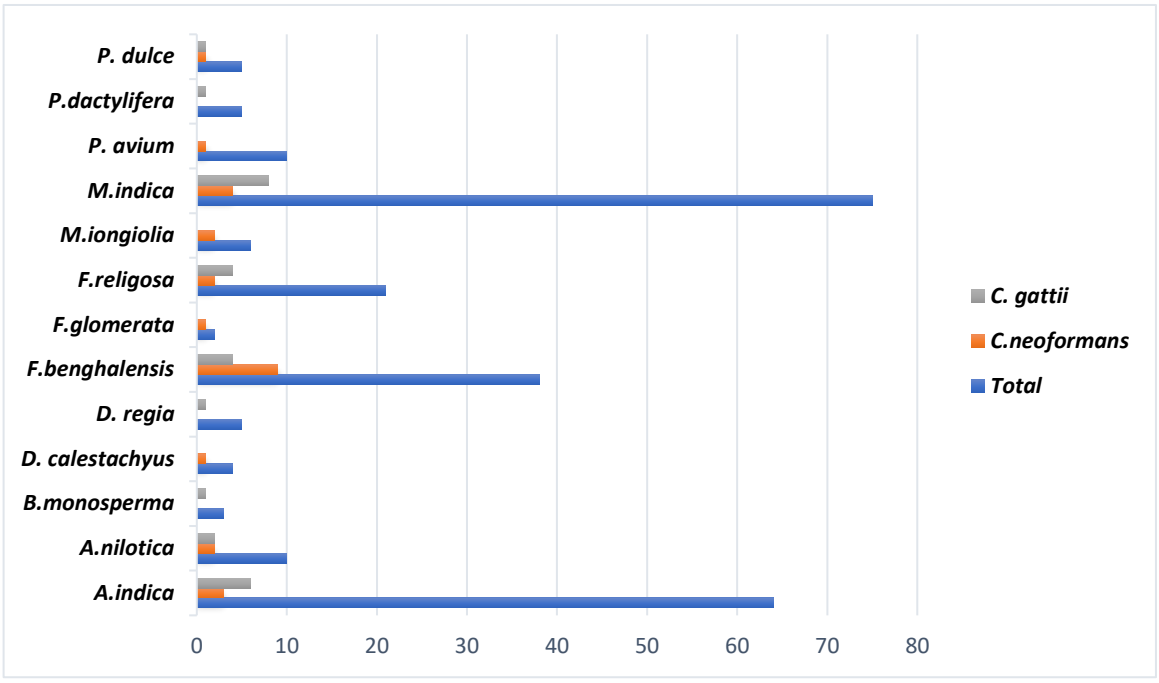


Figure 1- Showing total number of samples found positive for *C. neoformans* and *C. gattii* strains isolated from environmental sources.

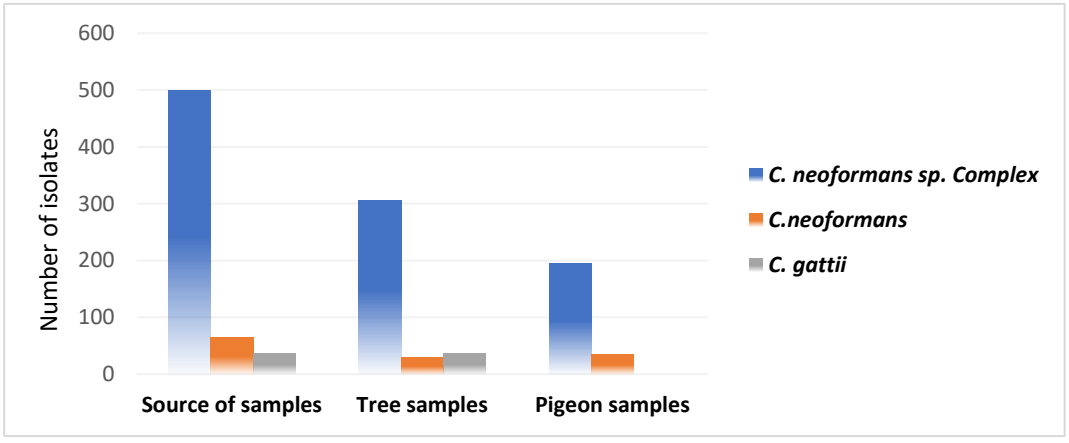
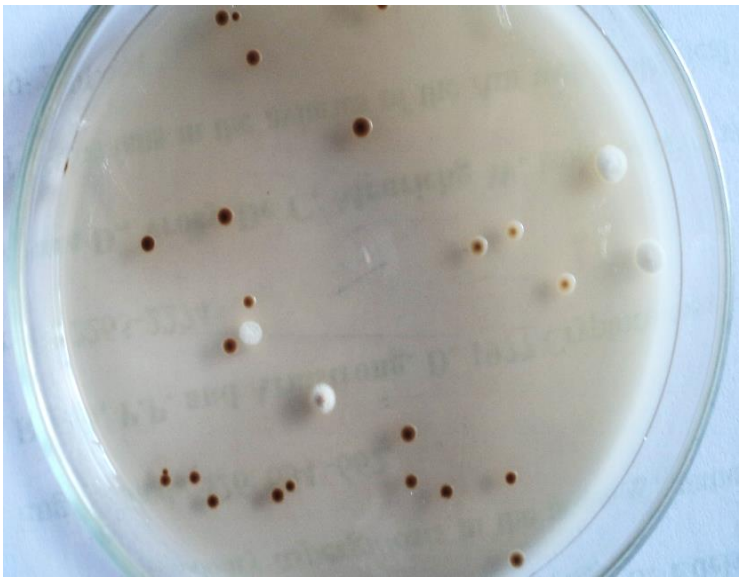
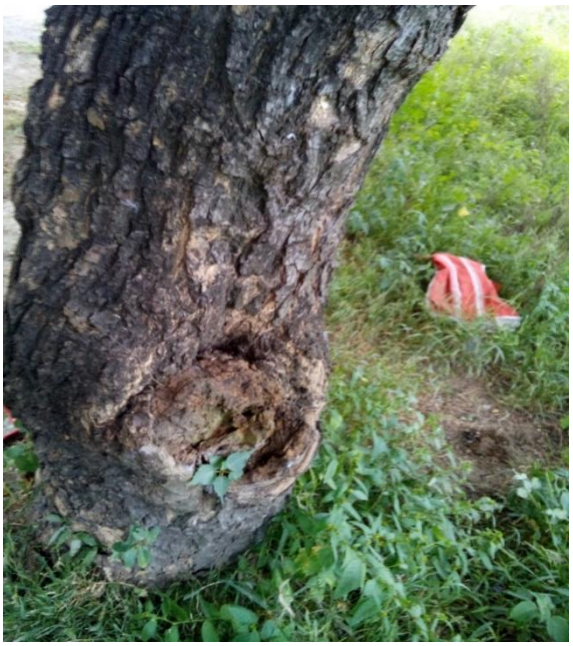


Figure 2- Showing total number of samples found positive for *C. neoformans* and *C. gattii* strains isolated from different tree sample

Figure 3: Showing isolation of *Cryptococcus* yeast from decaying wood of living tree trunk hollow. *Cryptococcus* yeast was producing brown colonies on staib (GACA) medium.





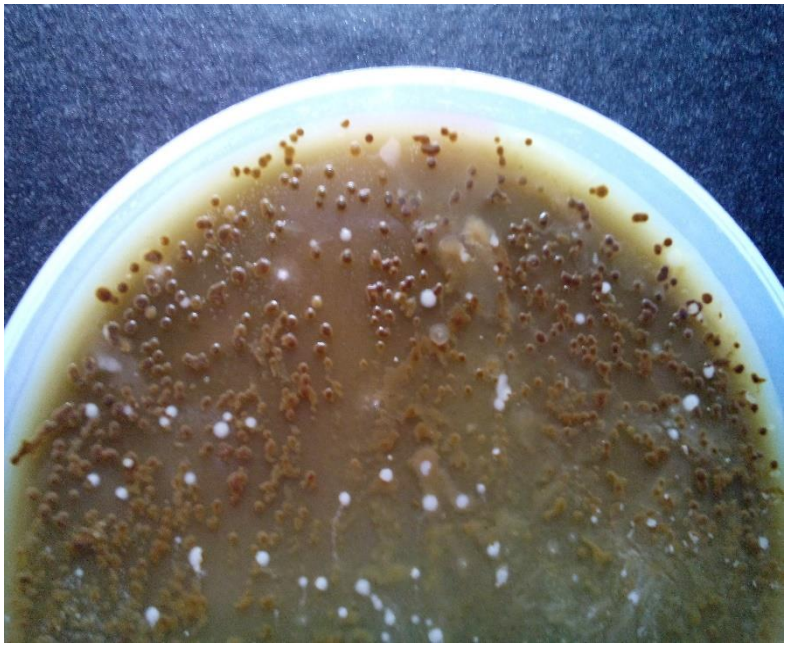


Figure 4: Showing isolation of *Cryptococcus* yeast from pigeon excreta samples. *Cryptococcus* yeast was producing brown colonies on staib (GACA) medium.

Significant reports established trees as the natural ecological niche of the pathogen as

per the various studies conducted in India. In accordance with the present study, Randhawa *et al.,* (2000) also revealed that *C. neoformans* dwell in *Tamarindus indica* tree and *Syzygium cumini* tree to inhabit both *Cryptococcus neoformans* and *C. gattii*.

Similar results were observed in some previous investigations including Grover *et al.,* (2007), who reported *C. neoformans* and *C. gattii* from, *M. indica, P. dulce, S. cumini*, and *T. indica*. Nawange *et al.,* (2006), who established the existance of *C. neo*formans species complex in *M. indica, S. cumini*, and *T. indica*.

Costa *et al.,* (2010), isolated *Cryptococcus neoformans var. neoformans* from pigeon droppings and confirmed the role of urban pigeons as a potential source of pathogenic yeasts. The yeasts can survive in moist or dry pigeon excreta for 2 years (Litvintseva *et al.,* 2005). Some studies strongly suggest zoonotic transmission of this yeast although it is difficult to link human infection to trees exposure (Lagrou *et al*., 2005).

Khan *et al.,* (1978) established a more frequent association of *C. neoformans* (55.4%) with old pigeon excreta in a Charity Birds Hospital, Delhi. Similarly, Pal *et al.,* (1989) reported *C. neoformans* from 38 (15%) of 253 old avian excreta samples. *C. neoformans* were isolated from 22 (34%) samples of droppings (Zarrin *et al.,* 2010).

Nawange *et al.,* (2000), for the first time reported the occurrence of *C. neoformans* from soils contaminated with pigeon excreta in Betul (M.P.). In which he examined 29 samples from which 9 were found positive and the highest frequency (75%) was recorded from Betul Bazar area.

In the present study, total 35 pigeon samples were found positive for *C. neoformans* and the highest frequency was observed from the pigeon sample collected from Bablu Talab Kothin Bazar (12.08%) which was contrary to the high frequency rate obtained by Soltani *et al.,* 2012 was 2.5%, Isfahani *et al.,* 2001 (8.1%) from Isfahan (Iran), and 60% frequency of *C. neoformans* by Gumasta *et al.,* (2019) from Jabalpur (M.P.).

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