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In the present study 225 pigeon droppings from two districts of West Bengal (Kolkata and North 24 Parganas) were collected. In total 24 (10.67%) *Cryptococcus neoformans* isolates were confirmed by phenotypical and biochemical characteristics. Minimum inhibitory concentration of the isolates was done by agar diffusion assay against fluconazole, ketoconazole, itraconazole, voriconazole and amphotericin-B. *C. neoformans*isolates produced the normal MIC range against the tested antifungals except 2 isolates (8.3%) which showed resistance to fluconazole (MIC value 64 µg/ml). It indicated the growing trend of antifungal resistance against fluconazole

Key words: Antifungal resistance, *Cryptococcus neoformans,* pigeon, West Bengal

INTRODUCTION

Cryptococcus neoformans is a haploid encapsulated yeast causing meningitis in immunosuppressed human and central nervous system disorders in a wide variety of animals such as dogs, cats, horses, cattle, goats and sheep. The most important pathogenic species are *C. neoformans-C. gattii* species complex which includes *C. neoformans* var. *neoformans*, *C. neoformans* var. *grubii* and *C. gattii* (*C. bacilli- sporus*). Sometimes interspecies hybrids of *C. neoformans* and *C. gattii* are observed (Samanta, 2015). The fungus is saprophytic in nature and ubiquitous in its distribution and has been recovered from a diverse type of environmental materials such as soil, bat guano, fruits, vegetables and wood (Chowdhary *et al,* 2012). Emmons first isolated *C. neoformans* from pigeon nests with their droppings containing uric acid and creatinine which provide enrichment for the growth of *C. neoformans.*

In India, Cryptococcal meningitis was reported as most common opportunistic infection in immunosuppressed patients. Among the domestic and pet animals occurrence of *C. neoformans* was recorded from mastitis in dairy cattle, cutaneous lesion and meningitis in cats. Like other parts of the world pigeon excreta was identified as a source of Cryptococcal infection in Northern and Western India. However the carrier status of pigeon in eastern India is still unexplored. So the present study was undertaken to investigate the occurrence of *C. neoformans* in pigeon excreta in selected area of West Bengal. The study was also intended to detect minimum inhibitory concentration (MIC) of common antifungals against the isolates.

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MATERIALS AND METHODS

Sampling

A total of 225 pigeon excreta (both old and fresh from each location) were collected from buildings, household pet bird shelters and aviary shops at different places in Kolkata and North 24 Parganas during 2013-2014. The samples were collected with wooden spatulas and were transported into the laboratory in sterile zippers.

Isolation and identification of Cryptococcus

About One gram of sample was suspended in 9 ml of sterile physiological saline (0.85%) supplemented with chloramphenicol (10 mg/ml). The mixture was left at room temperature for about 15-20 min, shaken manually for 4-5 min and incubated at 37! for one hour. 1 ml of supernatant from the suspension was inoculated in bird seed agar (HiMedia, India) and Sabouraud dextrose agar (SDA) with chloramphenicol (0.05 mg/ml) and incubated at 25oC and 37ºC, respectively (Pal et al., 1978). The plates were observed daily up to 4 weeks before discarding them as negative. *C. neoformans* was identified by colony characteristics in bird seed agar and SDA, urease positive character, negative for lactose assimilation, and negative reaction on CGB (Canavanine-Glycine-Bromothymol Blue) agar. Microscopic appearance of the isolates was studied by negative staining with India ink.

MIC determination

MIC of *Cryptococcus* isolates was done by agar diffusion assay using antifungal EzyTM MIC Strips (HiMedia, India) against fluconazole, ketoconazole, itraconazole, voriconazole and amphotericin-B (Abdel-Aal *et al*, 2007).

RESULTS AND DISCUSSION

The recovery of *C. neoformans* from the pigeon excreta in Kolkata and North 24 Parganas established that avian habitats serve as saprobic reservoir for this opportunistic pathogen. A total of 24 (24/225, 10.67%) *C. neoformans* isolates were obtained from 225 samples collected (Table 1). The positive samples of pigeon droppings were mostly dry, old and were not exposed to sunlight (Table 1). All the isolates produced characteristics brown coloured colonies in bird seed agar and mucoid colonies in Sabouraud dextrose agar. The positive isolates hydrolyzed urea and failed to utilize lactose. When examined by India ink stain under light microscopy the positive cultures showed circular, thinly encapsulated cells with and without budding. None of the isolates were able to change the colour of CGB agar medium while growing on it which confirms the isolates as *C. neoformans*.

A wide range of *C. neoformans* occurrence (5.2%-25.5%) was detected in different avian excreta throughout the world (Lugarini *et a*l, 2008; Hedayati *et al,* 2011). In India occurrence of *C. neoformans* was noted in old pigeon excreta specially in Delhi and Pune also.

C. neoformans isolates produced the normal MIC range against the tested antifungals except 2(8.3%) isolates which showed resistance to fluconazole (MIC value 64 µg/ml, Table 2). It indicated the grow-

Table 1 : Distribution of *Cryptococcus neoformans* isolated from pigeon in West Bengal

Location	*C. neoformans* isolates					
	Sample no.	Dry droppings	Moist droppings	Inside nests	Outside nests	Total of *Cryptococcus neoformans var. neoformans* (%)
Buildings	96	15 (15.6%)	1 (1.04%)	14	2	16(16.67%)
Household pet bird shelters	82	6 (7.32%)	1 (1.3%)	7	0	7 (8.54%)
Aviary shops	47	1 (2.13%)	0	0	1	1 (2.13%)
Total	225	22 (9.78%)	2 (0.89%)	21 (9.33%)	3 (1.3%)	24 (10.67%)

ing trend of antifungal resistance against fluconazole.

Table 2 : Determination of MIC against common antifungals

No. of isolates	Antifungal Agents	MIC range
Cryptococcus neoformans (24)	Fluconazole	1-64
	Ketoconazole	0.03-0.25
	Itraconazole	0.03-1
	Voriconazole	0.03-2
	Amphotericin-B	0.03-2

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REFERENCES

Abdel-Aal, A.M., Taha, M.M., El-Mashad, N. and El-Shabrawy, W. 2007, Antifungal susceptibility testing: new trends. *Egyptian Dermatol Online J.* **3:**1-10.

Chowdhary, A., Randhawa, H.S., Prakash, A. and Meis, J.F. 2012, Environmental prevalence of *Cryptococcus neoformans* and *Cryptococcus gattii* in India: an update. *Crit. Rev. Microbiol.* **38**: 1-16.

Hedayati, M.T., Mayahi, S., Fakhar, M., Shokohi, T. and Majidi, M. 2011, Cryptococcus neoformans isolation from swallow (Hirundo rustica) excreta in Iran. *Rev. Inst. Med. Trop. Sao. Paulo.* **53:** 125-127.

Lugarini, C., Goebel, C.S., Condas, L.A., Muro, M.D., de Farias, M.R., Ferreira, F.M. and Vainstein, M.H. 2008, *Cryptococcus neoformans* isolated from Passerine and Psittacine bird excreta in the state of Paraná, Brazil. *Mycopathol.* **166:** 61-69.

Samanta, I. 2015, *Veterinary Mycology*. 1st Edn., Springer.