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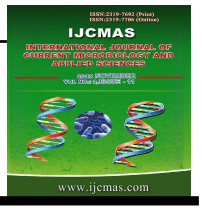

Original Research Article

Burden of *Candida* spp. and *Aspergillus* spp. in pond water in and around South Kolkata, India

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A B S T R A C T

The main objective of this study was to analyze the predominance of *Candida* spp. and *Aspergillus* spp. in pond water in and around South Kolkata. Although there are many studies on pond water regarding bacteriological microbiota, however, only a few studies are there on fungal population of pathogenic varieties in pond water which may cause infection in human beings. In this study we selected randomly thirty five ponds in South Kolkata nearby public and workplaces under the Kolkata Municipal Corporation and estimated Candida and Aspergillus population in water of those selected ponds following standard procedures of sample collection, processing, cultivation and identification of these species. In the collected pond water samples *Candida* spp. and *Aspergillus* spp. were detected in 52% and 34% respectively. Thus pond water of this locality may become a possible transmission route of these microorganisms to human beings and may be a potential health hazard, mainly to immunocompromised individuals.

Keywords

Aspergillus spp;
Candida spp;
Pond water;
Fungal enumeration.

Introduction

Fungi are widely distributed throughout the environment with a consignment of more than 70,000 species of them. Although fewer than 300 fungal species have been residing in human beings and less than a dozen cause about 90% of all fungus infections in general population, severe infection due to *Candida* spp. and *Aspergillus* spp. have recently been increased due to their super tolerance of growing to a physiological extremes as commmensals in different parts of body, most preferably in oral cavity (Coleman *et al.*1993), nasal cavity (Jarvis 1995), bronchus and gastrointestinal tract (Repentigny *et al.* 2000), genitalia (Ferrer 2000), skin (Koh *et al*, 2008) etc. Consequently they have been showing their outstanding infectivity in different forms of diseases ranging from mucosal to life threatening disseminated infections by activities of their fungal antigens, toxins, or direct invasion of the hosts. The invasive bloodstream

Candida infections (Pfaller and Diekema 2007) are associated with significantly higher rates of morbidity and mortality in immunocompromised hosts, and specifically infections of endogenous origin are associated with mortality rates up to 33-55%. Candidasis are frequently coupled with abrogated host immunity such as critically ill patients as a result of viral infections, cancer (Wingard 1995) human immunodefficiency virus (Badiee and Alborzi 2011), haematological and hormonal disorders, surgical procedures such as organ transplants (Baillie and Douglas 1999) , cytotoxic therapies with prolonged neutropenia, other immmunosupppresive therapies, use of corticosteroids and antitumor agents, antibiotic usage, indwelling invasive medical devices such as catheters and intensive care supports etc. *Candida* spp express different critical traits for existence on mucosal surfaces, where a unvarying but dynamic interplay occurs between innate and acquired host defence mechanisms. The virulence factors of these pathogens have been manifested by swift changes in phenotype, hyphal formations, molecular mimicry, biofilm formation (Finkel and Mitchell 2010) and ability to make enzymes, proteinasaes (Naglik *et al* 2003) and phoshpholipases (Niewerth and Korting 2001).

The imperative phenotypic changes of specific *Candida* spp include its ability to switch between different morphological forms - single celled, budding yeast form (blastospore) or in a filamentous form (including both pseudohyphae and true hyphae); Phoshpholipase effectively degrade phoshpholipids on cell surface; Proteinases destroy some important immune proteins such as immunoglobulin, lactoperoxidase, lactoferrin, mucin. proteinase activity additionally cause proteolysis of host surface proteins that leads to tissue damages. The capacity of *Candida* spp to rapidly acquire resistance to antifungal drugs, such as amphotericin B, flucytosine , and a series of azoles, demands the continued development of new antifungals (Enjalbert *et al*, 2009).

Candida albicans survives for long periods as a uniculture in sterile water or seawater. In the present study the other opportunistic animal and plant pathogen, *Aspergillus* spp. is ubiquitous in nature with remarkable adaptive behavior forming complex plant polymer in food stuff even with acidic pH, dried foods and those with high concentration of sugars such as jams, jellies; as for example many members of *A. glaucus* able to grow at low water activity and have been found on salted dry fish also. For the similar adaptation, grains, nut, spices are also attacked by xerophillic *Aspergillus* spp. Numerous species of the genus contaminate grains and other foods with toxic metabolites that becomes growing cause of threats to human, birds and other animals life . These saprophytes are copiously grown on organic compost piles, mouldy hay etc. They are capable to degrade starches, hemicelluloses, celluloses, pectins, other sugar polymers, fats, oils, chitin, keratin. In addition to these, they can grow in large numbers on papers and textiles (cotton, jute and linen). *Aspergillus* spores in aerosols, driftetd on air currents, dispersing themselves both short and long distances, resulting spores come in contact with a solid or liquid surface, they are deposited there and under favorable condition of moisture they germinate.

Aspergillus spp. can root animal disease in three major ways: Through the production of mycotoxins (aflatoxin) present in large

quantities in cassava, chillies, rice, wheat, corns, millet, cottonseeds, sunflowers seeds, pea nuts, tree nuts, sorghum and spices and also found in processed food stuffs such as meat, egg ,milk etc. Ingestion of these contaminated foods leads to hepatic disease, aflatoxicosis (Henry *et al*, 2002) that lead to edema and hemorrhagic necrosis of the liver with profound lethargy in human. Aflatoxin B1 is a potent carcinogen; secondly through the induction of allergic response, allergic response to *Aspergillus* spp to atopic individuals caused by inhalation of airborne spores is a common event which leads to hypersensitivity reaction such as asthma, hay fever (allergic rhinitis). High concentration of aspergillus spores inhalation leads to farmers lung, malt workers lung, bird fancier's lung disease and also leads to extrinsic allergic alveolitis (Salvaggio 2006) ; thirdly, through localized and systemic infection - Different forms of Aspergillosis like fungus ball formation (aspergilloma) where molds colonizes usually without spreading, causing granulomatous disease of lungs, and another is systemic aspergillosis (invasive infection) in which fungus disseminates throughout the body. Clinical manifestation and severity of the aspergillosis reflect the great concern on immunological status of the patient. Immunosuppressive agents and other medical practices have created ecological slot for *Aspergillus* spp for profound growth on immunocompromised host (Maschmeyer *et al*, 2007). Invasive aspergillosis is increasing in case of organ transplantation and bone marrow recipients.

As there are only a few studies on fungal population of pathogenic varieties in pond water which may cause infection in human beings we were interested to look into these aspects in thirty five ponds located in South Kolkata and estimated candida and aspergillus population in pond water of those selected ponds

Materials and Methods

Water samples

Water samples from randomly selected ponds nearby public and workplaces were collected from the Ward Numbers 92, 93, 102, 103, 104, 105 and 106 under the Kolkata Municipal Corporation. Thirty five ponds were selected. The samples for microbiological analysis were collected in sterilized plastic bottles and transported to the laboratory in ice box. Analyses were carried out within four hours of sampling. The ponds were considered in two categories, one was used and another was of unused categories. For used pond water, samples were collected from used side and also from their opposite sides. For unused ponds, water sample were collected from nearby roadside and its opposite sides.

Fungal enumeration

After collection water samples were centrifuged at 1200 rpm for 10 minutes and then the deposits were streaked on Sabouraud dextrose agar (SDA), supplemeted with streptomycin (50µg/ml). The plates were incubated at room temperature (~25^{O}C) and examined daily for one week.

Isolation of *Candida* spp and *Aspergillus* spp.

Colonies on SDA were subcultured on SDA for further identification of *Candida* and *Aspergillus* spp. by standard microbiological procedures.

Table.1 *Candida* and *Aspergillus* spp. in pond water (For Unused ponds)

Serial No	Sample Code	Sample address details	Near road side (cfu/ml).		Opposite of road side (cfu/ml).	
			Candida	*Aspergillus* spp	*Candida* spp	*Aspergillus*
1.	23	Jadavgar Horisava.	6	0	6	0
2.	28	Jadavgar Horisava.	4	1	1	0
3.	34	Asutosh Colony	27	0	0	0
4.	35	Suchetanagar	1	1	1	20
5.	12	B. A. Vidyalaya Rd.	2	1	4	0
6.	14	Nelinagar, Haltu.	3	0	0	0
7.	16	Nelinagar, Haltu.	37	1	14	0
8.	22	Jadavgar	2	3	10	0
9.	39	Jadavpur Lake	2	5	0	7
10.	43	Jadavpur University	3	0	0	3

Table.2 *Candida* and *Aspergillus* spp. in pond water (For used ponds)

S. No	Sample Code	Sample address details (Used, U1)	For used side (cfu/ml).		For unused side (cfu/ml).	
			Candida spp	*Aspergillus spp*	*Candida* spp	*Aspergillus* spp
1.	17	Dhakuria	1	0	0	0
2.	20	Ghoshpara, Haltu.	2	0	0	0
3.	40	Jadavgar pukur.	6	0	0	3
4.	31	K.P.Roy Lane.	6	0	0	0
5.	6	NeemtalaTarapeeth	6	0	5	3
6.	9	Ganguly pukur.	6	0	0	0
7.	26	Ghoshpara, Haltu	2	1	0	6
8.	24	Jadavgar (3, No).	3	2	8	4

Results and Discussion

Out of thirty five pond water samples eighteen showed positive culture result for *Candida* spp and twelve were positive for *Aspergillus* spp. (Table 1-2). Among them *Candida* spp. were found positive on one side and both side for ten and six unused ponds water samples respectively. Similarly *Candida* spp. were found positive in eight and two on one side and both side for used ponds water samples respectively. On the contrary *Aspergillus* spp were found positive on one side and both side for eight and two unused ponds water sample respectively and similarly they were found positive on one side and both side for four and two used ponds water sample respectively. Two samples (5·7 %) with *Candida* spp on SDA plate showed aberrant colony count (27 and 37 colony forming units/ml) and one sample (2·8%) with *Aspergillus* spp. also demonstrated deviant colony count (20 colony forming units/ml).

Presence of *Candida* and *Aspergillus* spp. was investigated in water samples of thirty five randomly selected ponds located in South Kolkata, West Bengal, India as these microorganisms are opportunistic pathogens for human beings. The study revealed their presence in significant numbers with 52% samples were positive

for *Candida* spp. and 34% samples were positive for *Aspergillus* spp.

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