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Short Communication

Seasonal Variation and Size Distribution in the Intramural Fungal Spore Concentration of Residential House in Bengaluru and their role in Respiratory Allergy

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Abstract

Indoor airborne fungal spores are known to have a significant role in the etiology of respiratory allergies. With the objective of identifying the indoor aeromycoflora and subsequently test the identified fungi on patients of respiratory allergy, investigations were carried out for a period of one year from March 2016 to February 2017 in the bed-room of suspected fungal allergy patient. We studied the seasonal variation and size distribution of fungal spore load in the bedroom of an old residential cum commercial area in north Bengaluru, Karnataka, India. During the period, weekly Petri plate exposures of Martin's Rose-Bengal Streptomycin agar media revealed a total of 1120 fungal colony forming units (CFUs). The samplings illustrates highest number of CFUs species in winter. *Aspergillus niger* exhibited the highest prevalence followed by *Penicillium chysogenum* and *Candida tropicalis*. The study comprises the first molecular characterization of a rare, new strain of *Candida tropicalis* isolated from the bedrooms. This sequence has been submitted to the GeneBank and accession number received for the same.

1. Introduction:

The major portion of the airspora consists of airborne fungal spores. These airborne fungal spores have a very significant role in the cause of respiratory allergy. 70% to 85% of the airspora is made up of fungal spores [1]. Most of fungi cause asthmatic and allergic problems, due to their differential deposition in the respiratory system. Inhalation of airborne allergic spores is responsible for initiating sensitization reactions which will lead to the respiratory allergic diseases.

Intramural aeromycology deals with airborne fungal particles inside close systems (buildings, apartments, houses, offices, libraries). Decrease of building ventilation for the purpose of space conservation and the fact that people spend majority of their time in indoors, exposes them to greater duration and concentration of indoor bioparticles [2]. Although Bengaluru is popularly known as the "Garden city of India", it has a sizeable portion of residents suffering from respiratory allergic disorders. The most frequently encountered disorders are allergic asthma and allergic rhinitis. Allergy prone individuals show an increase in their symptoms in Bengaluru.

2. Materials and Methods:

2.1. Study site:

The study site was the ground floor of a house in Malleshwaram, in the northern part of Bengaluru. The house measures 850 sq feet, bedroom measures 100 sq feet.

2.2. Samplings:

Three petriplates (of same size) containing MRBA (Martin's Rose Bengal Streptomycin Agar) medium were carried to the study sites in sterilized container and exposed to the bedroom air for standardized ten minutes to receive the of air borne fungal spores on the media plates. The exposure time was standardized to ten minutes to get countable number of fungal colonies/colony forming units per plate. After exposure and sealed with parafilm, Petri plates from site were brought separately to the Mycology Lab, Department of Botany, Joseph's college, Bengaluru.

3. Result and Discussion:

3.1. Qualitative and Quantitative Variations of the Aeromycoflora:

Aeromycological survey was carried out during 2016 to 2017 at Malleshwaram (Table 1; appendix 1). The results of the one year aeromycological survey showed that the bedroom was never free from fungal spores. The fungi varied with season and weather. A total of 37 fungal species were recorded from the set.

The incidence of fungal Colony Forming Units (CFUs) of the Site (Malleshwaram) for 2016-2017 (Table 1) has been provided. During the study period, 1120 CFUs were formed. The seasonal variations in the concentrations of CFUs were reported (figure 1). The percentage of CFUs was higher in winter (41.43%) and it was the lowest during summer (28.39%). The highest monthly count was reported in December with a total of 140 CFUs and the lowest count (38 CFUs) was reported in the month of March.

Aspergillus niger constituted the first highest fraction of the CFUs of the bedroom atmosphere with an annual incidence 214 CFUs (19.11%). *Penicillium chrysogenum* (137 CFUs, 12.23%), *Candida tropicalis* (72 CFUs 6.43%) was the third dominant among the airborne CFUs. *Cladosporium cladosporioides* (70 CFUs, 6.25%) was the fourth dominant fungus, whereas *Cladosporium bruhnei* (106 CFUs, 3.42%) occupied the fifth dominant place. *A. silvaticus* (61 CFUs, 5.45%), *Candida albicans* (60 CFUs, 5.36%), *Rhizopus* nigricans (55CFUs, 4.91%), *Alternaria alternata* (54CFUs 4.82%) and *Curvularia clavata* (43 CFUs, 3.84%) were the other prevalent dominant CFUs at the Site.

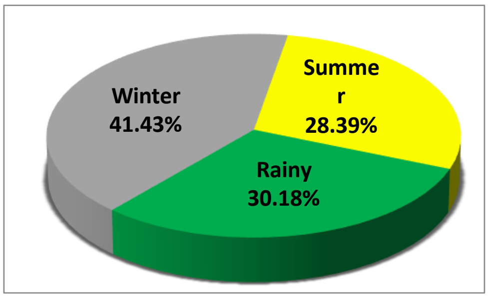
The above dominant fungi were sporadic in their occurrence at the site. Dry fungal spores like *Cladosporium, Alternaria* and *Aspergillus,* are saprophytic fungi. So their concentration depends mainly on the availability of plant debris and the velocity of the wind which release these spores. As reported in this study, Swaebly and Christensen (1952) [3] also reported that *Aspergillus* and *Penicillium* were predominant indoors, where as *Cladosporium* and *Alternaria* were most frequently monitored outdoors. The predominance of *Aspergillus* and *Penicillium* was reported by Dingmeng *et al* (2020) [4]. Amir *et al* (2017) [5] reported that *Cladosporium* and *Candida* were the frequently indoor monitored genera. *Cladosporium* and *Candida* were also found to be dominant in the present study.

3.2.Seasonal Variations of Aeromycoflora:

Site had December 2016, November 2016 and February 2017 as high CFU months, whereas the number of CFUs recorded was low in March 2016, May 2016, and August 2016. From the above results, it is obvious that the CFUs concentration was not uniform throughout the year. Seasonal variations of aeromycoflora were also reported [6],[7],[8],[9]. Winter has shown dominance of CFUs (41.43%). Winter which begins with the end of the North-East Monsoon favors greater growth of fungi. The moisture provided by the tail end of the monsoon and cooler months of the winter help in the proliferation of fungi and their dispersal. The fungal spores are normally dispersed from the conidiophores by hydrostatic pressure and wind. The summer stood in least dominance of CFUs (28.39%). The dry months of summer with the paucity of moisture inhibits fungal growth. The results of the present study are in conformity with Jurado *et al*, (1990) [10]; Kuo and Li, (1994) [11] and Kuder (2003) [12] and Rodriguez *et al*, (2005) [13]. The highest count of *Penicillium* *chrysogenum, Candida tropicalis* (Figure 2 and 3) and *C. albicans* was registered in winter. The summer season showed the same fungal types as seen in winter, but had much lesser numbers (Figure 1).

3.3. Confirmation of Airborne Fungi by Molecular Identification:

Identification of one dominant species of fungi was confirmed by 18S rDNA sequencing. GenBank Accession Numbers were obtained from NCBI (National Center for Biotechnology Information) for the selected dominant fungus. This technique was adopted when doubts about their species level persisted even after pure culture. The identity of the species which were confirmed was *Candida tropicalis* (Figure 4) (Accession number: MH879817). The study showed *C. tropicalis* to be one of the common aerospora, where as *Candida* species marginally less investigated by other workers.



4. Conclusion:

In this study, it is found that *Aspergillus* is the most common indoor fungi present at the indoor environment. The finding correlates with the results of Agashe and Anuradha (1998) [14], Dharmage *et al.*, (2002) [15], and Michael *et al.,* 2016 [16]. In environments, such as tropical habitat, these fungi are dominant and are known for their allergenicity [17]. This study indicates that more moisture, less temperature with lower hygiene resulted in high airborne fungal concentration.

Figure 1: Percentage distribution of CFUs based on seasons (March 2016-February 2017).

Figure 2: Pure culture of *Candida tropicalis* (pure culture-Streak method).

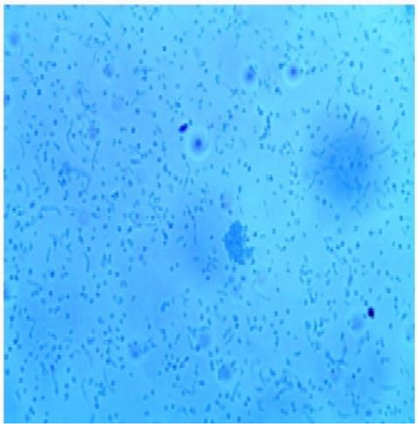


Figure 3: *Candida tropicalis* (Microscopic view)

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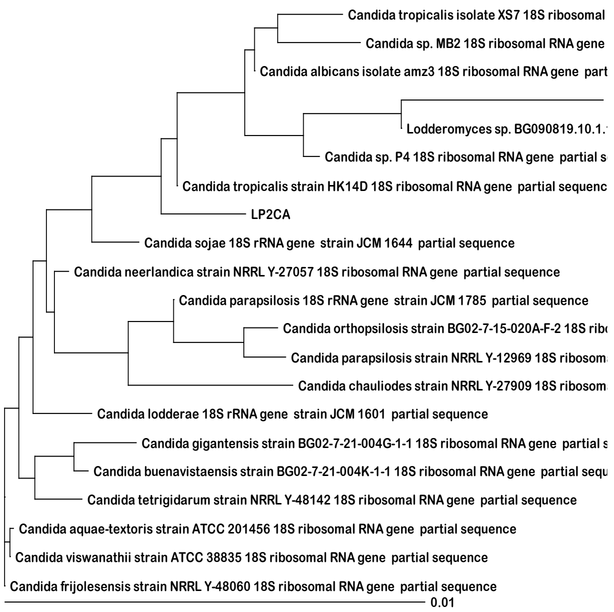

Figure 4: Phylogenetic tree of *Candida tropicalis* with the GenBank Accession Number MH879817

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Appendix 1

Table No. 1- Indoor aeromycological survey carried out from the Site-1 during the years 2016 - 2017

Sr. NO	FUNGI	MAR. 16	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB. 17	TOTAL NO.OF CFUs	% OF CFUs
1	*Aspergillus niger*	1	51	20	81	2	1	15	9	25	2	3	4	214	19.11
2	*Penicillium hrysogenum*		1	0	17	18	11	18	14	33	4	13	8	137	12.23
3	*Candida tropicalis*	5		1	1	1		10	1	10	16	8	19	72	6.43
4	*Cladosporium ladosporioides*		1		1	26		18	19		3	2		70	6.25
5	*Cladosporium bruhnei*	1		2		7	19	4	28		2	1		64	5.71
6	*Aspergillus silvaticus*	5	2	9	1		8		14	16	1	2	3	61	5.45
7	*Candida albicans*	7			3		3	2		8	14	8	15	60	5.36
8	*Rhizopus nigricans*		3	6	2		2	9	5	9	5	4	10	55	4.91
9	*Alternaria alternata*	1		2	1		3	5		11	12	7	12	54	4.82
10	*Curvularia clavata*	4	1	9	3	1		2		5	9	8	1	43	3.84
11	*Helminthosporium sativum*	3		6	2		1	9	1		12	5	2	41	3.66
12	*Epicoccum andropogonis*					2			3		17	4	13	39	3.48
13	*Fusarium solani*		2		3		4	2	6	5	3	3	7	35	3.13
14	*Trichoderma viride*	1	1			1	1			2	7	5	8	26	2.32
15	*Aspergillus ochraceus*		1		5		2	1		4	9		1	23	2.05
16	*Aspergillus terreus*		13		6		2							21	1.88
17	*Alternaria crassa*	2	2		6			1	2		2	1	1	17	1.52
18	*Fusarium oxysporum*	2	1		2	1					3	4	2	15	1.34
19	*Alternaria solani*						2	1			9			12	1.07
20	*Aspergillus flavus*	2	1		3	1					1	3	1	12	1.07
21	*Aspergillus tamarii*						2	1			9			12	1.07
22	*Penicillium oxalicum*			4	2									6	0.54
23	*Fusarium moniliforme*					4		1						5	0.45
24	*Sporothrix schenckii*	1				3						1		5	0.45
25	*Hyaline mycelia*					1	1		1	1				4	0.36
26	*Alternaria citri*					1			1					2	0.18
27	*Aspergillus candidus*									2				2	0.18
28	*Mucor mucedo*											2		2	0.18
29	*Penicillium citrinum*	1					1							2	0.18
30	*Pithomyces graminicola*											2		2	0.18
31	*Aspergillus glaucus*	1												1	0.09
32	*Aspergillus ustus*								1					1	0.09
33	*Aspergillus versicolor*	1												1	0.09
34	*Aureobasidium pullulans*					1								1	0.09
35	*Lichtheimia corymbifera*			1										1	0.09
36	*Paeceilomyces varioti*			1										1	0.09
37	*Penicillium glabrum*							1						1	0.09
	Total number of CFUs	38	80	61	139	70	63	100	105	131	140	86	107	1120	