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Allergenic Significance of Air-borne Fungal Spores of Allergy Patients' Residences

B P SINGH*, P K MUKERJEE** and P NATH
Department of Pathology and Bacteriology, King George's Medical College, Lucknow

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Air-borne fungal spores were isolated and identified using culture plate method to find out the common offending fungal allergens in houses of allergy patients. *Aspergillus*, *Cladosporium*, *Penicillium*, *Alternaria* and *Curvularia* have shown significant abundance in most of the houses, alongwith some other fungal types. *Aspergillus* had shown its maximum abundance (39.3%) affecting about 89.3% houses. A correlation has been observed between fungi present in houses of allergy patients and skin sensitivity to allergenic extracts of same fungal types in the patients.

Key Words: Allergenic significance, Fungal spores, Allergy patients, Residences

Introduction

The role of air-borne fungal spores in aetiology of respiratory allergy is well established (Prince & Morrow 1969). The findings of Kramer in 1932 and 1940 have revealed that fungi growing in houses were a prime factor in causing asthmatic symptoms in low-lying humid regions. Further, a correlation has been found between fungi growing in houses of patients and skin reactivity to extracts of same fungal genera found in the houses (Jones & Gerson 1971). Therefore, the first and foremost prerequisite for the appropriate diagnosis and management of respiratory allergy is to identify the air-borne fungal spores of both in-door's and out-door's.

Accordingly, the present study was undertaken to investigate the prevalence of various air-borne fungal spores in-door's of allergy patients of Lucknow and to find out the skin sensitivity to the allergenic extracts of isolated fungal types in allergy patients.

Materials and Methods

Exposure of culture plates: The houses of 113 allergy patients who attended the "Allergy Clinic" during 1978 in the

**Present address*: Antigen Division, Centre for Biochemicals, VP Chest Institute Building, University Campus, Delhi 110007
***Department of TB and Chest Diseases, KG's Medical College, Lucknow

department of T B & Chest Diseases, King George's Medical College, Lucknow were visited for the exposure of culture plates. These houses were situated in different localities of the city namely, Chowk Thakurganj, Husainabad, Aminabad, Husainganj, Sadar Bazar and Mahanagar. On the basis of the general appearance, the houses were mainly of two types (I) Old, with earthen floor, damp and closed and (II) New with concrete floor dry and (open) ventilated. In bedroom of each patient one culture plate (10 cm diameter) containing Rose-Bengal-Streptomycin-agar medium was exposed for 5 min at the height of 1 meter above the ground level.

The bedrooms of patients were chosen as site for exposure of culture plates as the bedrooms are most lived by the patients (Lumpkins, Corbit & Tiedman 1973). For a general and preliminary screening of air-borne fungal spores, exposure of culture plates was done only once in each house after an hour of raising the house dust at about 9 AM (Maunsell 1954).

Identification and Isolation of fungi: The exposed culture plates were incubated at 25°C to 32°C for sufficient period for proper growth of fungal colonies. The fungal colonies developed were identified, counted and isolated. The subcultures were also prepared and sent to Comonwealth Mycological Institute, Kew, England for authentic identification.

Preparation of allergenic extracts: Fifteen fungal types which were found frequently harbouring in houses of allergy patients were selected for extraction. These were mass cultured using Beiberdorf and Aurgabrite-broth medium (Maunsell 1954) and harvested after proper sporulation. The dried, powdered fungal spore mass was used for the preparation of allergenic extracts in 1:50 W/V (2%) concentration in phosphate-buffred-saline (Sheldon Lovell & Mathews 1967).

Clinical studies: Intradermal skin tests were carried out (Shivpuri 1962) on 54 patients, out of the total 113 allergy patients whose houses were screened for air-borne fungal spores. They were of different age groups and suffering with various allergic disorders like Bronchial Asthma, Allergic Rhinitis, etc. The skin tests on each patient were carried out with all the 15 fungal extracts which were isolated from their house as well as with others which were not.

In addition as a control, intradermal skin tests were also done on 20 healthy non-allergic volunteers to check the possibility of any non-specific-irritants in the antigens.

Results

The details of prevalence of various fungi in houses of allergy patients are given in table 1.

A total number of 2395 fungal colonies belonging to 17 fungal types were identified in all the 113 culture plates (about 21 colonies/plate/house) exposed and studied. Though, no qualitative variations were observed in fungal flora of type-I and type-II houses, the fungal flora in type-I houses was observed more abundant (about 25 colonies/plate/house) in comparision to type-II houses (about 15 colonies/plate/house) (table 1).

In most of the houses, *Aspergillus* spp have shown their maximum abundance: 39.3% followed by *Cladosporium* spp 19.4%, *Penicillium* spp 21.1%, etc. Some of the fungal types, e.g., *Aureobasidium pullulans*, *Phoma hibernica*, *Trichothecium roseum*, etc. have represented below 1% in-door's mycoflora. Frequency of almost all the fungi was found higher in type-I houses excluding *Alternaria* spp and *Helminthosporium* spp which were found in higher concentrations in type-II houses.

The detailed results of skin sensitivity tests with allergenic extracts of 15 fungi

Table 1 *Prevalence of various air-borne fungi in house of allergy patients of Lucknow city*

S.No.	Fungi	Type- I houses=62 Fungal colonies		Type-II Houses=51 Fungal colonies		Total houses=113 Fungal colonies	
		No.	% abundance	No.	% abundance	No.	% abundance
1.	*Aspergillus* spp	679	42.9	263	32.3	942	39.3
2.	*Cladosporium* spp	301	19.02	166	20.4	467	19.4
3.	*Penicillium* spp	208	13.08	93	11.3	301	12.1
4.	*Alternaria* spp	94	5.9	106	13.03	200	8.3
5.	*Curvularia* spp	81	5.1	42	5.1	123	5.1
6.	*Fusarium* spp	50	3.1	42	5.1	92	3.8
7.	*Mucor pussilus*	39	2.08	21	2.5	60	2.5
8.	*Epicoccum purpurascens*	30	1.8	13	1.5	43	1.7
9.	*Helminthosporium sativum*	22	1.3	23	2.8	45	1.8
10.	*Rhizopus oryzae*	22	1.3	10	1·2	32	1.3
11.	*Paecilomyces fusisporus*	16	1.01	12	1.4	28	1.1
12.	*Aureobasidium pullulans*	14	0.82	4	0.49	18	0.75
13.	*Phoma hibernica*	11	0.69	4	0.49	15	0.62
14.	*Trichothecium roseum*	7	0.44	3	0.36	10	0.41
15.	*Trichoderma longibrachiatum*	3	0.18	5	0.61	8	0.33
16.	*Cunninghamela elegans*	2	0.12	5	0.61	7	0.20
17.	*Myrothecium roridum*	3	0.18	1	0.12	4	0.16
	TOTAL	1582		813		2395	

**Specific details*: Species arranged in order of (higher to lower) abundance in-doors

1. *Aspergillus flavus* (11%) abundance, *A. niger* (9%), *A. fumigatus* (9%), *A. sydowi* (3%), *A. nidulans* (2%), *A. versicolar* (1%), *A. terreus* (1%) and *A. tamari* (below 1%);
2. *Cladosporium cladosporioides* and *C. oxysporum*;
3. *Penicillium funiculosum, P. islandicum* and *P. cyclopium*;
4. *Alternaria alternata* and *A. humicola*;
5. *Curvularia lunata, C. clavata* and *C. tuberculata*;
6. *Fusarium solani* and *Fusarium semitectum*

isolated from in-door's of patients are given in table 2.

The markedly positive reactions in the patients to extracts of same fungal types which were isolated from their houses ranged from 16% to 44%. On the contrary, the markedly positive reactions in the patients to extracts of fungi which were not found in their houses ranged from 2.8% to 15.0%. None of the 20 healthy volunteers tested with same fungal extracts, showed any markedly positive skin reactions. However, 1⁺reactions were not uncommon (table 2).

For comparing skin reactions, only markedly positive skin reactions (2⁺ to 4⁺) were taken into consideration as they are

Table 2 *Account of skin sensitivity tests with extracts of fungi isolated from indoors*

S. No.	Fungi	Total Allergy Patients=54						Healthy controls=20	
		Patients showing fungi in houses	Patients showing +ve skin sensitivity (2+ to 4+)		Patients not showing fungi in houses	Patients showing +ve skin sensitivity (2+ to 4+)		Skin sensitivity* (1+ only)	
		No.	No.	%	No.	No.	%	No.	%
1.	*Penicillium funiculosum*	34	15	44.0	20	3	15.0	3	15
2.	*Aspergillus fumigatus*	27	11	40.7	27	4	14.8	4	20
3.	*Aspergillus terreus*	10	4	40.0	44	2	4.3	5	25
4.	*Fusarium solani*	15	5	33.3	39	3	7.6	3	15
5.	*Helminthosporium sativum*	12	4	33.3	42	5	11.9	3	15
6.	*Rhizopus oryzae*	6	2	33.3	48	1	2.8	2	10
7.	*Cladosporium cladosporioides*	32	10	31.2	22	3	13.5	3	15
8.	*Aspergillus sydowi*	11	3	27.2	43	4	9.3	3	15
9.	*Curvularia lunata*	17	4	23.5	37	2	5.4	2	10
10.	*Aspergillus niger*	23	5	21.7	31	1	3.2	5	25
11.	*Aspergillus flavus*	28	6	21.4	16	2	12.5	6	30
12.	*Aspergillus nidulans*	5	1	20.0	49	—	—	2	10
13.	*Mucor pussilus*	5	1	20.0	49	1	2.04	2	10
14.	*Alternaria alternata*	25	4	16.0	29	2	6.8	1	5
15.	*Apergillus versicolar*	6	—	—	48	1	2.8	2	10

*There were no markedly positive reaction (2+ to 4+) observed in healthy volunteers; +ve=positive

likely to contain fewer false positive skin reactions (Shivpuri & Agarwal 1971).

Discussion

The predominance of a certain species or group of fungi at any particular indoor site would appear to be determined by properties of immediate environment (Richards 1954). Certain saprophytic fungi which have been observed in higher frequencies in-doors because the atmosphere inside the houses may be more conducive for their growth, e.g., *Aspergillus*, *Penicillium*, *Cladosporium*, etc. On the other hand, certain fungal types, e.g., *Alternaria* and *Helminthosporium* which thrive mostly as plant pathogens and were observed frequently in out-doors (Vishnu Mittre & Khandelwal 1973) were observed expectedly in lower frequencies in-doors (table 1).

However, the prevalence of these two fungal types, namely, *Alternaria* and *Helminthosporium* was found higher in type-II houses as compared to type-I houses due to the fact that the type II houses were well ventilated and were with concrete floors where the fungal flora is expected to be more or less the same as of out-doors.

The precentage of markedly positive reactions in patients to the extracts of same fungi which were isolated from their houses was observed higher in comparison to those which were not isolated from their houses (table 2). This is in full agreement with the findings of Jones and Gerson (1971).

Although, this study was confined only to the indoor mycoflora but when compared with the results of the outdoor fungal flora reported by Vishnu Mittre and Khandelwal (1973), present data showed wide variations both qualitatively as well as quantitatively. Hence, it is recommended that for proper diagnosis and management of respiratory allergy patients of a given geographical area, both in-door's as well as out-door's, fungal flora should be recorded to prepare a complete list of the fungal types to which the patients are exposed during each 24 hr.

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*Not seen in original