

Aeromycological Survey of Delhi: Significance in Management of Respiratory Allergic Patients

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ABSTRACT

Introduction: Aeroallergens are a significant cause of respiratory morbidity and mortality, particularly in patients with respiratory allergic disorders such as asthma and rhinitis. Understanding the prevalence of local aeroallergens is critical for accurate diagnosis and effective treatment. Airborne fungal spores, one of key component of aeroallergens, vary significantly with geographical and seasonal factors. This study focused on detecting and quantifying airborne fungal spores in New Delhi to better understand their contribution to respiratory allergic disorders.

Aim: To analyse the prevalence and seasonal variations of airborne fungal spores in New Delhi through systematic air sampling.

Materials and Methods: The present cross-sectional study was conducted for one year at Vallabhbhai Patel Chest Institute, New Delhi, India and air sampling was conducted over 69 systematic sampling days by vertically installed Air Sentinel device on the institute's main hospital building roof to capture airborne fungal spores. Fungal spores were collected, their species identified through microscopic examination, and specific allergen levels quantified using immunochemical methods. Seasonal variations in fungal spore prevalence were analysed, along with the correlation between total and viable spore counts.

Results: A total of 217,982 fungal spores were collected, with *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Epicoccum*, and *Drechslera* identified as the predominant airborne species. Significant seasonal variations were observed in both total and viable spore counts. Immunochemical analysis demonstrated notable seasonal fluctuations in specific fungal aeroallergen levels; however, no significant correlation was found between these levels and total or viable spore counts with Rank correlations (Batschelet; 1981).

Conclusion: This study highlights the significant seasonal variations in airborne fungal spore prevalence in New Delhi, India with *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Epicoccum*, and *Drechslera* identified as the predominant species. While specific fungal aeroallergen levels fluctuated seasonally, they showed no significant correlation with total or viable spore counts. These findings highlight the importance of continuous air quality monitoring and targeted allergen assessment to better understand the impact of fungal spores on respiratory allergic disorders, guide targeted interventions, and improve patient outcomes in high aeroallergen exposure regions.

Keywords: Air pollution, Asthma, Environmental allergen

INTRODUCTION

Environmental allergen exposure is a well-established factor that not only contributes to the development of new allergic sensitivities but also exacerbates existing allergic conditions, thereby increasing overall morbidity [1]. Accurate diagnosis and effective treatment of respiratory allergic disorders require a thorough understanding of the prevalence and seasonal pattern of various local allergens. Exacerbation of allergic symptoms in fungal-allergic patients is more strongly associated with specific allergenic fungal spore concentrations rather than total fungal spore counts [2].

Numerous aerobiological studies have been undertaken across various regions of the country to determine the airborne concentrations and seasonal patterns of pollen grains and fungi [3]. A variety of sampling devices, each with its own advantages and limitations, have been employed to capture particles through gravity, impaction, and suction. The collected air samples can be analysed to evaluate total fungal spores, both viable and non-viable, as well as specifically viable spores. However, no single method provides comprehensive and reliable data on the presence of both viable and total fungal spores in the air. Furthermore, these techniques do not accurately represent the actual airborne fungal allergen load [4]. The aim of the study was to analyse the prevalence and seasonal variations of airborne fungal spores in sampling and to evaluate the correlation between total fungal spore counts and immunochemical quantification of specific fungal aeroallergens.

The present study identified and enumerated airborne fungi in New Delhi, India examined the impact of meteorological factors (temperature, relative humidity, and rainfall) on their seasonal variations, and quantified the fungal allergen content for selected genera.

MATERIALS AND METHODS

A cross-sectional observational study was conducted at the Vallabhbhai Patel Chest Institute for one year, New Delhi, India. A volumetric suction sampler (Air Sentinel) was mounted vertically on the rooftop of a seven-storey hospital building, approximately, 20 meters above ground level. The sampler was positioned in an open area without surrounding walls or obstructions to ensure unimpeded airflow. Airborne particles were collected on Polytetrafluoroethylene (PTFE) membranes (2.5 cm diameter, pore size=0.3 µm; Quan-Tech Air Inc., Rochester, MN, USA) at an average flow rate of 42 liters per minute. No protective gear was placed around the sampler during operation, as the elevated location and air suction pressure prevented insects, reptiles, or other contaminants from interfering with the sampling process. However, the sampler was intermittently covered when not in use to protect it from environmental factors.

Air sampling was conducted biweekly over a continuous one-year period, with samples collected on three consecutive days during each sampling interval. The PTFE membranes were replaced every two months to ensure consistent sampling efficiency. Culture plates were not directly exposed during the sampling process. After

collection, the exposed PTFE membranes were analysed for total spore counts and colony identification. This methodology ensured reliable and consistent monitoring of airborne particulate matter over an extended period, providing valuable data for further analysis.

Study Procedure

Processing of air samples:

- Viable airborne fungal spores: Each PTFE membrane was immersed in 10 mL of sterile 0.1M NH_4HCO_3 (pH 7.8). Aliquots of 100 μL each were spread plated in dilutions onto petri dishes containing Rose Bengal Streptomycin Agar medium. Following appropriate incubation, the developed colonies were identified, counted, and isolated [3-8]. The concentration of viable fungal spores per cubic meter of air was subsequently calculated.
- Procedure for airborne allergens elution: Particles were eluted for 72 hours at 4°C, after which the suspension was centrifuged. The resulting sediment was reconstituted in 1 mL of distilled water, and 10 μL of this suspension was mounted on a slide. Spores were then identified and counted using direct microscopy, and the concentration of allergens per cubic meter of air was calculated.
- Quantification of airborne allergens by Radioallergosorbent Test (RAST) assay: Supernatants from three consecutive days were pooled, centrifuged (1000 rpm for 10 minutes), and lyophilised. Each lyophilised sample was reconstituted in 1 mL of RAST diluent. Aliquots of 100 μL from the reconstituted samples were used as liquid-phase inhibitors RAST assay for three fungal species: *Alternaria alternata*, *Epicoccum* sp, and *Fusarium chlamydosporium*. Allergen levels in the test samples were determined based on the inhibition lines produced with homologous fungal extracts in the RAST inhibition assay.

The PTFE membrane was extracted from the suspension and inspected under a microscope to confirm the effective removal

observed, with peak spore concentrations occurring between February and May, followed by a secondary peak in October.

The most predominant spores were round spores 16,578 (43.7%), primarily representing the genera *Aspergillus* and *Penicillium*. These were followed by multicellular (septate) conidia, including *Cladosporium* spp. 10,370 (27.4%) and *Alternaria* spp. 2,956 (7.8%). Other identified spores included *Epicoccum* spp. 1330 (3.5%), *Drechslera* spp. 818 (2.3%), *Ascospores* 553 (1.5%), and *Curvularia* spp. 481 (1.4%), collectively accounting for 87.6% of the total airborne spores captured [Table/Fig-1].

The seasonal distribution of significant fungal types, as determined through direct microscopy and culture techniques, exhibited a consistent pattern, with peak concentrations aligning between February and May across both detection methods [Table/Fig-2].

- Viable fungal counts: Over the one-year sampling period, a total of 4,873 fungal colonies were identified using culture techniques after incubation in Rose Bengal Streptomycin Agar medium, with an average daily count of 71 viable fungal spores per cubic meter of air. The most common fungal genera detected were *Aspergillus* 238 (28.1%), *Cladosporium* 210.7 (24.9%), *Alternaria* 149.4 (17.6%), *Penicillium* 43.9 (5.2%), *Curvularia* 28.9 (3.4%) and *Epicoccum* 27.9 (3.3%). Significant seasonal variations were observed in the viable spore counts of different fungi [Table/Fig-2,3]. A comparison of the daily mean concentrations per cubic meter of total and viable spores for major types of spores is presented in [Table/Fig-2,4].
- Aerial fungal spore counts and meteorological factors: The study compared total fungal spore counts with various meteorological factors, such as rainfall, relative humidity, and sunshine duration, revealing significant variations in fungal growth patterns, as illustrated in [Table/Fig-5].

S. No.	Fungal spores↓	Month→	Feb	Mar	Apr.	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Annual	
															Total	%
1	Round spores		1198	8203	1035	1729	333	380	429	592	1194	563	543	379	16578	43.7
2	<i>Cladosporium</i>		1168	878	1138	904	532	391	609	404	1237	916	1484	709	10370	27.4
3	<i>Alternaria</i>		127	386	591	758	148	202	118	100	227	95	92	112	2956	7.8
4	<i>Epicoccum</i>		97	349	315	225	29	54	23	26	87	36	43	46	1330	3.5
5	<i>Drechslera</i>		15	75	123	95	46	25	89	91	130	52	47	30	818	2.3
6	<i>Ascospores</i>		33	49	23	40	25	132	104	8	85	22	26	6	553	1.5
7	<i>Curvularia</i>		20	40	26	28	46	55	33	78	107	14	11	23	481	1.4

[Table/Fig-1]: Daily mean concentration of total prevalent spores/ m^3 of air (Direct microscopy) in Delhi area using Air Sentinel.

of particles. No membrane displayed a significant number of remaining particles. The immunochemically measured allergen content of the three fungal allergens was compared with their respective colony (viable) and spore (total) counts, as determined by rank correlation.

Amount of allergen/ m^3 of air was calculated by formula:

Allergen content in 100 μL of air sample eluate:

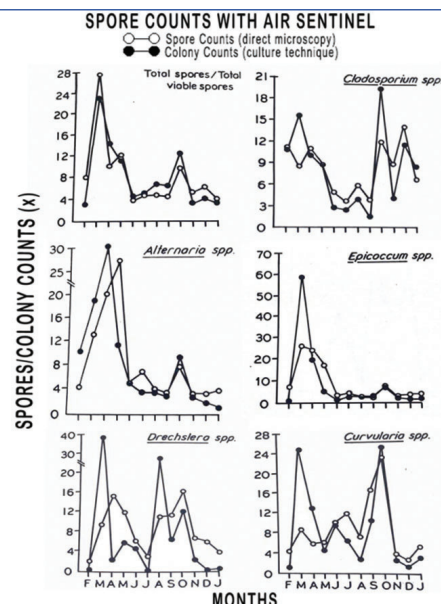
181.44 (Total air sampled in 3 consecutive days) 42 $\times$ 60 $\times$ 72 hours

STATISTICAL ANALYSIS

Meteorological data were obtained from the Meteorological Department of Delhi, India. The collected data was analysed using GraphPad as the statistical tool. Rank correlation was assessed.

RESULTS

- Total spore counts: Using the air-sentinel, a total of 217,982 fungal spores were captured over 69 systematic sampling days, yielding an average daily concentration of 3,159 fungal spores per cubic meter of air. Notable seasonal variations were



[Table/Fig-2]: Viable spore count with air sentinel.

S. No.	Fungal type↓	Month→	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Annual	
															Total	%
1	<i>Aspergillus</i> spp.		9.9	57.3	34.7	27.4	7.9	9.9	12.2	26.5	31.4	9.3	5.5	6	238	28.1
2	<i>Cladosporium</i> spp.		23.2	33.1	21	18.8	6	5	8.4	3.2	41.2	8.7	24.5	17.6	210.7	24.9
3	<i>Alternaria</i> spp.		15.2	28.1	46.5	16.9	7.1	5	5	4	13.7	3.7	2.5	1.7	149.4	17.6
4	<i>Penicillium</i> spp.		1.7	13.7	5.4	4.7	0.8	0.3	4.7	3.4	3.2	2.8	1.7	1.5	43.9	5.2
5	<i>Curvularia</i> spp.		0.3	7.1	3.6	1.2	2.6	1.7	0.7	2.9	7.2	0.6	0.3	0.8	28.9	3.4
6	<i>Epicoccum</i> spp.		0	16.1	5.4	1.2	0.3	0.6	0.6	0.6	1.8	0.6	0.4	0.3	27.9	3.3

[Table/Fig-3]: Daily mean concentration of dominant viable spores/m3 of air (culture technique) in Delhi area using Air Sentinel.

S. No.	Spore	Month	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Annual	%
															Total	
	Total trapped															
	Spore		2994	10358	3785	4564	1447	1755	1764	1633	3673	1928	2428	1581	37910	
	Colony		52.1	195.1	119.7	93.1	37.9	40.4	57.1	54.5	105	28.1	35.7	28.7	847.4	
Major types of fungal spores																
	Cladosporium															
	Spore		1168	878	1138	904	532	391	609	404	1237	916	1484	709	10370	27.4
	Colony		23.2	33.1	21.0	18.8	6.0	5.0	8.4	3.2	41.2	8.7	24.5	17.6	210.7	24.9
	Alternaria															
	Spore		127	386	591	758	148	202	118	100	227	95	92	112	2956	7.8
	Colony		15.2	28.1	46.5	16.9	7.1	5	5	4	13.7	3.7	2.5	1.7	149.4	17.6
	Curvularia															
	Spore		20	40	26	28	46	55	33	78	107	14	11	23	481	1.4
	Colony		0.3	7.1	3.6	1.2	2.6	1.7	0.7	2.9	7.2	0.6	0.3	0.8	28.9	3.4
	Epicoccum															
	Spore		97	349	315	225	29	54	23	26	87	36	43	46	1330	3.5
	Colony		0	16.1	5.4	1.2	0.3	0.6	0.6	0.6	1.8	0.6	0.4	0.3	27.9	3.3
	Drechslera															
	Spore		15	75	123	95	46	25	89	91	130	52	47	30	818	2.3
	Colony		0	4.7	0.3	0.7	0.6	0	3.4	0.8	1.5	0.3	0.1	0.1	12.5	1.5

[Table/Fig-4]: A comparison of daily mean concentration/m³ of major types of total (Direct microscopy) and viable spores (culture technique) in the air samples collected by Air-Sentinel.

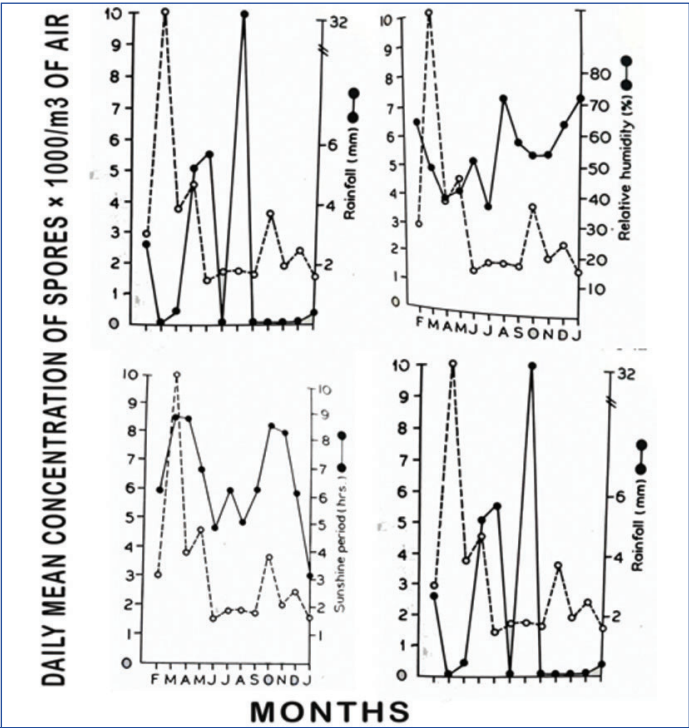
S. No.	Allergen	Range (ng/m ³)	Peak periods
1	<i>Alternaria alternata</i>	1.5 - 2894.2	May and October
2	<i>Epicoccum</i> sp.	0.9 - 115.8	March and April
3	<i>Fusarium chlamydosporium</i>	9.4 - 49.8	May and October

[Table/Fig-5]: Summarised results of Immunochemical quantification of various airborne fungal allergens.

4. Immunochemical quantification of fungal aeroallergens: Significant seasonal variations were observed in the airborne allergen content of immunochemically quantified fungal allergens. However, no notable correlation was found between the allergen levels of the three test fungi and their total or viable spore counts. The immunochemically quantified allergen content varied from 1.5-2894.2 ng/m³ of air [Table/Fig-6].

DISCUSSION

The intricate relationship between respiratory allergic disorders and local aeroallergens is a multifaceted issue that varies significantly with environmental conditions and seasonal changes. As highlighted by Masoli M et al., the onset and severity of allergic symptoms are intimately linked to the concentrations of various aeroallergens, including pollen, fungal spores, and animal dander [9]. This connection emphasises the critical need for comprehensive studies that explore the types, prevalence, and seasonal fluctuations of these airborne particles, as established in earlier research [10-14]. Such studies are vital for developing effective management strategies and improving patient outcomes.



[Table/Fig-6]: Daily mean concentration of spores.

Historically, methods for assessing allergen exposure have relied heavily on direct counting of identifiable particles and colony enumeration from air-exposed culture plates. However, Agarwal MK

et al., identified significant limitations within these traditional approaches, particularly regarding their effectiveness in correlating allergen counts with patient symptoms outside the typical pollen and fungal seasons [10]. The conventional techniques often fail to capture the total airborne allergen load, which can result in a misleading understanding of a patient's allergenic environment. This gap in knowledge has prompted the exploration of more advanced methodologies, including immunochemical quantification, which allows for a more comprehensive assessment of allergen exposure regardless of the source [10]. This advancement represents a crucial shift in allergen analysis, paving the way for a more nuanced understanding of the factors contributing to respiratory allergies.

Additionally, the interaction between airborne allergens and air pollution further complicates this narrative. Although numerous studies have suggested a correlation, a meta-analysis by Lam HCY et al., found that only about one-third of these studies were able to establish a significant relationship between allergens and air pollution in the onset of respiratory conditions [15]. This inconsistency highlights the need for more detailed and comprehensive research that can effectively disentangle the complex dynamics at play, potentially involving synergistic effects that exacerbate allergic responses.

In a position paper on aerobiology and clinical aspects of mold allergy it has been pointed out that there is no single technique which gives a complete and reliable information about the presence of both viable and total fungal spores in the air [16]. Moreover, as the culture plates can be exposed only for short period, they do not give counts representing all the 24 hours.

In the context of India, the landscape of aeroallergens is particularly diverse and presents unique challenges. Recent research has documented 83 pollen species, 34 fungal types, and various other allergenic entities that contribute to significant allergen exposure, both indoors and outdoors [17]. A striking 82.2% of patients with allergic rhinitis were found to be sensitised to at least one allergen, underscoring an urgent need for effective management strategies tailored to the specific allergens prevalent in the region [18]. Our modified method has also been used for the quantification of viable fungal spores. Again, significant correlation ($r=0.66$) was obtained between the total spores (Direct microscopy) and viable counts (culture technique) by this technique. Significant correlations were observed between spore & colony counts of majority of fungal types *Cladosporium* ($r=0.85$), *Alternaria* ($r=0.89$), *Drechslera* ($r=0.71$) barring a few, *Epicoccum* ($r=0.48$). This could be due to presence of viable hyphae of *Epicoccum* and/or some non-viable *Epicoccum* spores in air.

Despite advancements in detection technologies, such as automated devices for real-time pollen identification, spore trap sampling with microscopy remains the most widely employed method for assessing airborne allergens due to its reliability [19]. However, this approach is not without its challenges. It requires trained personnel to operate effectively and suffers from limitations such as the restricted exposure time of culture plates, which often fail to capture the full spectrum of airborne fungi.

In response to these limitations, the present study introduced a novel air sampling approach that facilitates the simultaneous quantification of total and viable fungal aerospora over a full 24-hour period. This method provides a significant improvement in the accuracy of allergen exposure assessment and aligns with previous research indicating the necessity of reliable volumetric estimates of aeroallergens [20]. The identification of dominant fungal spores—such as *Cladosporium*, *Alternaria*, and *Epicoccum*—reaffirms the validity of the sampling technique of current study as these findings are consistent with those of other studies in the field.

Moreover, the study results demonstrated significant correlations between total and viable spore counts, reinforcing the importance of accurately quantifying viable fungal spores in understanding allergen exposure. The distinct seasonal patterns observed in fungal spore

counts correlate with meteorological factors, particularly sunshine hours, which are known to influence spore release dynamics [21]. These correlations provide valuable insights into how environmental conditions shape allergen exposure, which can inform both clinical management and public health strategies.

The present study investigation into three clinically relevant fungal allergens—*Alternaria alternata*, *Epicoccum* sp., and *Fusarium chlamydosporium*—revealed that traditional spore and colony counts frequently do not accurately represent the actual airborne allergen load. This finding is consistent with earlier research that emphasises the necessity for immunochemical quantification in clinical allergy studies [10,22]. By adopting a multi-faceted approach that integrates various methodologies, we can develop a more comprehensive understanding of airborne allergens and their impacts on respiratory health.

Limitation(s)

The main limitations of this study include its single-location focus in New Delhi, India which may limit generalisability, and the lack of multi-site sampling with control locations to account for environmental variability. Biweekly sampling may have missed short-term fluctuations, and the absence of direct culture plate exposure limited real-time viability assessment. Reliance on culture and microscopy techniques may have underestimated non-culturable fungi. These factors highlight the need for broader, multi-site, real-time studies with advanced methodologies.

CONCLUSION(S)

Aeroallergens are important determinants of respiratory morbidity in Type I respiratory diseases. The identification of dominant fungal types by various effective methods simultaneously will help in generating a full proof data which will be utilised by the clinicians for proper diagnosis and treatment of local allergic patients. This is a significant information for the benefit of local people with such ailments, and will go a long way in prevention of unnecessary exposure, generation of appropriate battery of fungal allergens for diagnosis by skin testing and also for immunotherapy.

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