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Analysis of airborne microfungi in indoor environments of different hotels in Pondicherry city

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ABSTRACT

The fungal spores are unanimous in their distribution found indoors and outdoors of the hotel environment and constitute the major portion of the suspended bioparticulate matter in the air. An aeromycological survey of the two indoor sites viz., Kitchen and the Dining hall of five different hotels in Pondicherry city were carried out by employing volumetric Burkard's personal sampler using SDA mediated petridishes during 2015. Air samplings were made at intervals for isolating the prevalent fungi from the study sites in between 10 AM to 1PM. In total,10 media plates were utilized during the study period and altogether, 29 fungal species under 14 genera were isolated, among which Aspergillus sp. were recorded as the dominant followed by Penicillium sp. The prevalence of spores was found more in the Kitchen in comparison to the Dining hall of the hotels. Besides Aspergillus, other fungi like Absidia, Alternaria, Cladosporium, Curvularia, Fusarium, Monascus, Mucor, Paecilomyces, Penicillium, Rhizopus, Trichoderma, Verticillium and Wallemia were also recorded. The analysis of data indicated that concentration of airborne fungi in the two indoor sites mostly in Kitchen and Dining hall of the Hotel is very high and quite variable depending on the climatic conditions and substrate availability. The allergenic spores of Aspergillus and Penicillium were found to be predominant, probably due to their wide host range, substrate adaptability and opportunistic nature. The variation of the fungal types was highly related to the availability of the substrate precipitation needed for the fungal growth. The occupational variations and climatic alteration had positive/negative influence on occurrence of aeromycoflora in the indoor environments of the hotels studied herewith.

Keywords: Volumetric analysis, Airborne microfungi, Burkard's sampler, CFUs, Hotels

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INTRODUCTION

Air borne fungi are common in nature and their occurrence is very high in different working environments viz., bakeries, tanneries, poultries, sugar factories, vegetable markets, saw mills and dwellings where, organic raw materials support their growth and sporulation [1,2]. The estimation of the diverse spores present in these environments is an important objective for most of the allergists and food microbiologists [3]. Studies on airborne fungi in hotel environments are not available in India and very rare in abroad. However, Marvin [4] carried out a study in the Hilton hotel environment. He stated that the air quality issues at the hotel due to mold, the hotel has reviewed guidelines and recommendations published by OSHA and the Center for Disease Control to ensure that the hotel has been taking all appropriate measures to ensure the quality at the hotel in safe mode. Based on his work, he opined that the air quality at the hotel is safe and concluded that the Hotel has, both in the past and continuing through the present, taken effective measures consistent with the guidelines and recommendations published by

OSHA and the CDC to ensure that air quality at hotel is safe. The Hotel has followed and will continue to follow OSHA and CDC guidelines regarding the control of mold to provide safe and healthful air quality. The Hotel inmates are not aware of any evidence which would suggest anyone at the Hotel has experienced adverse health effects due to air quality at the Hotel. While a very small number of employees have reported that they suspect mold may be the cause of some of their personal health problems and at the present time the Hotel is not aware of any medical or other evidence to substantiate such suspicions. The effect of human activity on counts of viable microorganisms in indoor air is clearly shown by the converted Andersen sampler counts for the dining area of the restaurant, with the largest counts occurring during the busy lunchtime period. More recently an investigation was carried out for a group of Scottish houses [5], the median count of viable airborne fungi in the indoors, as determined by the CAMNEA method, was 260 colony forming units (CFU) m^{-3} air. In view of the above report, the present study was an attempt to record the viable airborne microfungi in indoors of different hotels in Pondicherry city volumetrically by Burkard's personal sampler on agar plates during 2015.

EXPERIMENTAL SECTION

Pondicherry is the capital city of Puducherry state situated 160 km away from Chennai on the south and it is in the coromondal coast of Bay of Bengal basically ruled by the French. Pondicherry is located in between 11 degree 46º and 12 degree 30 º of north latitude and between 79 degree 36 º and 9 degree 52º of east longitude. The layout of Pondicherry is located within Tamil Nadu which presents a peculiar picture of territorial jurisdiction perhaps the only one of its kind in the word.

An aeromycological study was carried out in the indoor sites, Kitchen and Dining hall of following five different hotels viz., 1) Srikarpaga Vinayagar Chettinad Hotel, 2) Billal Hotel, 3) Saravana Bhavan Hotel, 4) Buhari Hotel, 5) Virudhunagar Hotel in Pondicherry city during January to April 2015. The Hotels seems to be the favorite hangout for the present modern generation. Hotel is one of the most frequently visiting places for all the people and it also serves the needs of the common people in Pondicherry City. The Hotels that were selected for my study are situated in New Bus stand, Anna Salai, Lawspet and Kathirkammam area of the city and all these places are surrounded on all sides by market complexes, schools, colleges, residential quarters, air port, etc.

Air Sampling

The Burkard's Volumetric Air Sampler on agar plates was used in the present study. The air quality was analyzed by collecting airborne fungal bioaerosols directly onto the petriplates in the two Indoor sites, Kitchen and the Dining hall of the hotel environment. The Volumetric Air Sampler is designed for short-term sampling in domestic or industrial environments particularly where no power supplies are available. The Burkard's Volumetric Air Sampler is a perfect air quality monitor used in culture rooms, clean rooms, laminar flow cabinets and domestic environments for collecting fungi and other particles directly onto a glass slide for immediate microscopic observation. It was designed to record the total number of bioaerosols per cubic meter of air in the sampling sites.

Air samples for culturing fungi were collected by the Petri Plates supplemented with SDA medium (Sabouraud's Dextrose Agar) in the operating samplers. The sampler was run at the height (1.5-2m) above the ground just to the breathing level based on the substrates available in the Hotel. After operation, the Petri Plates were brought to the laboratory in the Pre-sterilized polythene bags and incubated at 25±3 ºC for 3-7 days. After three days of incubation, the fungal colonies were counted for individual species and the total number CFUs were calculated. Microscopic slides stained with lactophenol cotton blue were prepared from each CFUs and observed microscopically under the light microscope to identify directly them up to species level. The colony forming units (CFUs) that could not be identified directly from plates were sub cultured in SDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal taxa [6,7,8,9,10,11]. Cultured fungi on agar plates of different hotel sites and the identified fungal taxa up to their species level are given in tables. Percentage occurrence of individual fungus were determined and plotted in the form of tables and figures.

Calculation of Percentage contribution of an individual fungus:

$$\% \text{ occurrence of the fungus} = \frac{\text{Total CFUs recorded by the individual fungus}}{\text{Total CFUs recorded by total number of fungi}} \times 100$$

RESULTS

During the study period, on qualitative study, altogether 29 fungal species under 14 genera were isolated from the two indoor sites of the five different hotels. Out of the total 5460 fungal CFUs, indoors of the kitchen covered 68.2% of fungal spores and the dining hall had 31.8% of the fungal spores (Fig 1).Comparison of total number of fungal CFUs/m^{-3} isolated from different hotels environments is given in Fig 2, which showed that Sri Karpaga vinayagar chettinad hotel was dominated among all.

Fig 1: Occurence of total fungi in Kitchen and Dining hall of Indoor of Hotel in Pondicherry City

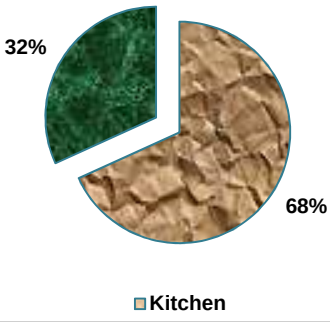


Fig 2: Comparison of spore profiles in two sites of Hotel environments

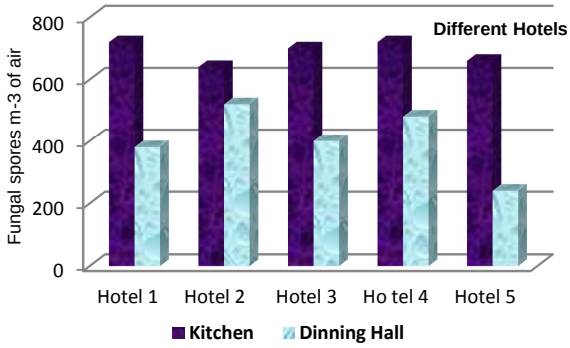


Fig 3: Percentage distribution of fungal spores in Dining hall of Hotels

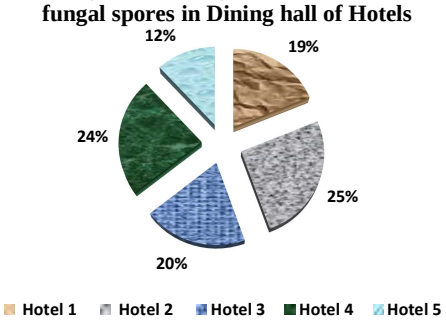
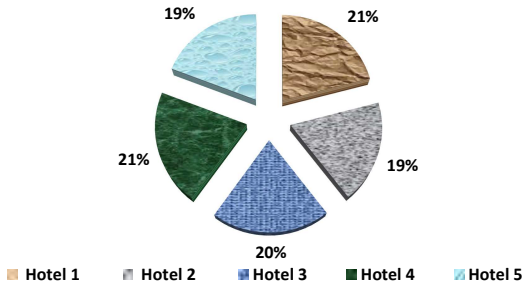


Fig 4: Percentage distribution of fungal spores in Kitchen of Hotels



In percentage occurrence, Fig 3 and 4 supported the difference in the incidence of fungal spores in the hotel sites. In analyzing the diversity of fungal species, both in two areas, the Kitchen site contributed the maximum number (24 species under 19 genera) and the Dining hall contributed 23 species of 11 genera but when it comes to the concentration of fungal load (spores) Kitchen was also dominated over the Dining hall. Among the recorded taxa, members of Deuteromycotina were most prominent in their occurrence followed by the members of Zygomycotina. Among all, *Penicillium chrysogenum* was found to be the dominant one in kitchen followed by *Aspergillus awamori, Cladosporium herbarum, Penicillium citrinum,* white sterile mycelia. *Aspergillus niger* was occurred as the dominant one followed by *Aspergillus awamori, Cladosporium herbarum, Penicillium chrysogenum* in the Dining hall (Table 1 and 2). Besides these *Aspergillus flavus, A. fumigatus and Penicillium digitatum* were recorded frequently from the hotel environments.

Altogether, ten species of Aspergilli were isolated i.e., *Aspergillus awamori, A. flavipes, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. restrictus, A. tamarii, A. terreus, Aspergillus sp*. Five species of *Penicillium* were also isolated in both the sites of hotel environments i.e., *Penicillium citrinum, P. chrysogenum, P. digitatum, P. expansum, P. oxalicum.*

Table 1: Percentage of the occurrence of air borne fungal spores m^{-3} of air inside the Kitchen of various hotels environment in Pondicherry city

Sl. No.	Name of the fungi	Hotel A	Hotel B	Hotel C	Hotel D	Hotel E
1	*Absidia sp.*	5.55	-	-	-	-
2	*Aspergillus awamori*	-	-	-	19.44	-
3	*Aspergillus flavipes*	11.11	-	-	-	-
4	*Aspergillus flavus*	8.3	-	-	5.55	-
5	*Aspergillus fumigatus*	-	-	-	5.55	-
6	*Aspergillus niger*	2.7	53.12	57.14	16.66	6.06-
7	*Aspergillus restrictus*	-	-	-	-	3.03
8	*Aspergillus tamarii*	-	-	-	-	-
9	*Aspergillus terreus*	-	-	-	-	3.03
10	*Aspergillus sp.*	13.8	-	-	-	-
11	*Cladosporium herbarum*	19.44	-	-	-	-
12	*Curvularia sp.*	-	9.37	-	-	-
13	*Fusarium oxysporum*	-	3.1	2.85	-	-
14	*Monascus sp.*	-	-	-	-	3.03
15	*Mucor racemosus*	-	3.1	8.57	-	-
16	*Penicillium citrinum*	-	-	-	16.66	39.39
17	*P.chrysogenum*	30.5	-	-	19.44	6.06
18	*Penicillium digitatum*	-	-	-	-	24.24
19	*Penicillium expansum*	2.7	-	-	2.77	-
20	*Penicillium oxalicum*	-	-	-	13.88	-
21	*Rhizopus sp.*	-	9.37	-	-	-
22	*Verticillium sp.*	2.7	-	-	-	-
23	White sterile mycelia	2.7	21.8	31.42	-	-
24	Yellow sterile mycelia	-	-	-	-	15.15

Table 2: Percentage occurrence of air borne fungal spores m^{-3} of air inside the Dining hall of various hotel environments in Pondicherry city

Sl. No.	Name of the fungi	Hotel A	Hotel B	Hotel C	Hotel D	Hotel E
1	*Absidia sp.*	-	-	5	-	-
2	*Alternaria alternate*	10.52	-	-	-	-
3	*Aspergillus awamori*	-	15.38	10	8.33	8.33
4	*Aspergillus flavipes*	-	3.84	-	-	-
5	*Aspergillus flavus*	10.5	3.84	-	4.16	-
6	*Aspergillus fumigatus*	-	3.84	10	4.16	-
7	*Aspergillus niger*	26.3	34.61	35	12.5	-
8	*Aspergillus ochraceus*	-	-	10	-	-
9	*Aspergillus tamarii*	-	-	5	-	-
10	*Cladosporium herbarum*	21.05	15.38	-	-	-
11	*Mucor racemosus*	-	-	-	4.16	-
12	*Paecilomyces sp.*	5.2	-	-	-	-
13	*Penicillium citrinum*	-	11.53	-	16.66	-
14	*P.chrysogenum*	10.5	7.69	20	8.33	58.33
15	*Penicillium digitatum*	-	-	-	4.16	8.33
16	*Penicillium expansum*	10.5	-	-	-	-
17	*Penicillium oxalicum*	-	-	5	12.5	8.33
18	*Rhizopus sp.*	-	-	-	-	8.33
19	*Rhizopus stolonifer*	5.2	-	-	-	-
20	*Trichoderma sp.*	-	-	-	-	8.33
21	*Verticillium sp.*	-	3.84	-	12.5	-
22	*Wallemia sebi*	-	-	-	4.16	-
23	White sterile mycelia	-	-	-	8.33	-

Among the isolated fungal taxa, *Aspergillus fumigatus, A. niger, Penicillium sp., Rhizopus stolonifer* were predominant aeroallergens that can cause different type of respiratory/lung diseases in atopic human beings. *Aspergillus fumigatus* causes broncho-pulmonary aspergillopsis diseases. *Aspergillus flavus*, a mycotoxin producing fungus was abundantly recorded from the two sites of the five hotels.

The fungal spores were recorded with the maximum in the kitchen site due to the abundance of aspergilli and penicilli.

In indoor (Kitchen), maximum number of species was obtained in Srikarpaga Vinayagar Chettinad Hotel viz., *Absidia sp., Aspergillus flavipes, Aspergillus flavus, Aspergillus niger, Aspergillus sp., Cladosporium herbarum, Penicillium chrysogenum, Penicillium expansum, Veticillium sp.,* and the least number of species obtained in the Saravana Bhavan Hotel viz., *Aspergillus niger, Fusaium oxysporum, Mucor racemosus.*

In Dining Hall, maximum number of species was obtained in Buhai Hotel viz., *Aspergillus awamori, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Mucor racemosus, Penicillium citrinum, Penicillium chrysogenum, Penicillium digitatum, Penicillium oxalicum, Verticillium sp., Wallemia sebi* and the least number of species obtained in the Virudhu Nagar Hotel viz., *Aspergillus awamori, Penicillium chrysogenum, Penicillium digitatum, Penicillium oxalicum, Rhizopus sp., Trichoderma sp.*

In the kitchen environments, *Aspergillus niger* was frequently occurred in comparison to other species and in the dining hall, *Penicillium chrysogenum* was frequently occurred in comparison to other species. White sterile mycelia were identified in dining hall and the Yellow sterile mycelia were identified in Kitchen.

In Kitchen sites, *Absidia sp., Aspergillus awamori, A. restrictus, A. terreus, Aspergillus sp., Cladosporium herbarum, Curvularia sp., Fusarium oxysporum, Penicillium oxalicum, Verticillium sp*., Yellow sterile mycelia were occurred less in number and the Dining hall region, *Absidia sp., Alternaria alternata, Aspergillus flavipes, A. ochraceus, A. tamarii, Mucor racemosus, Paecilomyces sp., Penicillium oxysporum, Rhizopus stolonifer, Rhizopus sp., Trichoderma sp., Wallemia sebi*, white sterile mycelia were occurred less in number.

DISCUSSION

The present study was carried out by employing Burkard's volumetric air sampler on agar plates at both the study sites of the hotels was found to be the best method in order to analyze the airborne fungal spores per cubic meter of air in comparison to petriplate exposure methods made by previous workers [12,13,14,15]. Irrespective of place, duration and technique of sampling the predominance of deuteromycetes fungi in air spore was confirmed by all the investigators [16].

Occurrence of aeromycoflora was recorded at two sites in five different hotel environments. It was found that the fungal spores were the maximum in the kitchen followed by the dining hall with minor difference. But the differences in fungal spore load in the Kitchen and Dining hall of the hotel were depended on the abundance of *Aspergillus niger* followed by *Penicillium chrysogenum*. The lesser Hotel activities were recorded in the indoor sites of the dining hall. The abundance of fungi more in the Kitchen site may be due to the dispersion of fungal mass from the substrates available in the kitchen and the relative humidity prevailing in the said environments. The hotel atmosphere was particularly dense in spores; this is certainly due to the fact that the extremely cramped locality was a site of intense activity and the moisture laden environment. The variations in the number of CFUs in different points of the hotels do not appear to be significant. In 60% of the cases, the atmosphere seemed to be heavier with spores in close proximity to the ovens [17].

A number of *Aspergillus* sp. (7 species) such as *A. flavipes, A. flavus, A. fumigatus, A. glaucus, A. japonicas, A. niger and A. sydowii* were reported to be of high incidence in the present study was found similar to the findings of Singh et al., [18]. According to Nayak and his coworkers [19], the high level of relative humidity in the atmosphere provokes the incidence of fungal spores in the air. It is the condensation form of water present on the surfaces, which favors the growth of aspergilli. *Aspergillus* spp. was the dominant fungi in the hotel environments studied by us. In our study, *Aspergillus niger* was found to be the dominant one in Dining hall followed by *Penicillium chrysogenum, Penicillium citrinum and Cladosporium herbarum.* The fungal spores of *Penicillium chrysogenum* was the dominant one in its occurrence followed by *Aspergillus niger, Penicillium citrinum, Penicillium oxalicum* in the Kitchen of the indoor site.

Health effects are associated with exposure to fungal spores and considered to be genus-specific. In an enclosed environment, fungal spores can become airborne in large concentrations and incite potential hazards of occupational

allergies or other lung disorders to atopic and sensitized individuals [20]. The large concentration of spores of *Aspergillus flavus* and *Aspergillus niger* and *Cladosporium* and *Penicillium citrinum* correlated with the higher prevalence of symptoms in workers during these period [21]. The large concentrations of *Aspergillus flavus* need a more detailed investigation in view of its possible role in lung disease [22].

CONCLUSION

The analysis of data indicated that concentration of airborne fungi in the two indoor sites mostly in Kitchen and Dining hall of the Hotel is very high and quite variable depending on the climatic conditions and substrate availability. The allergenic spores of *Aspergillus* and *Penicillium* were found to be predominant, probably due to their wide host range, substrate adaptability and opportunistic nature. The variation of the fungal types was highly related to the availability of the substrate precipitation needed for the fungal growth. The occupational variations and climatic alteration had positive/negative influence on occurrence of aeromycoflora in the indoor environments of the hotels studied herewith. The presence of microfungi in the environment emphasizes both allergists and biologists with an interest in health and environment bio pollution to solve the sufferings of the people who have allergenic disorders in the said environments. On account of the biological features and ease of dispersion of the fungal spores in both the indoors are considered to be one of the chief agents of contamination in all the five hotel environments, hence particular remedy would be taken care to minimize the fungal load in these environments.

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