

Isolation, identification and testing for allergenicity of fungi from air-conditioned indoor environments

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Abstract Twelve fungi were isolated and identified from air-conditioned rooms. Of the 12, 7 were species of *Aspergillus*, viz. *A. niger*, *A. oryzae*, *A. fumigatus*, *A. terreus*, *A. nidulans*, *A. versicolor* and *A. parasiticus*. Other fungi were *Penicillium citrinum*, *Fusarium oxysporum*, *Trichoderma viride*, *Neurospora crassa* and *Alternaria alternata*. *A. niger* was present in 80% of the locations. Some of the fungi isolated in this study could be opportunistic fungal pathogens, like *A. fumigatus*, *A. niger* and *Penicillium citrinum*, and were found to be allergenic. Results of this study indicate that air-conditioned rooms could be reservoirs of fungi and may cause allergic problems or infections in healthy or immunocompromised individuals living in these environments.

Keywords Allergenicity · Allergic ·
Aspergillus · *Penicillium* · Skin prick test

1 Introduction

With progress, advancement and development, a sizeable number of people stay in air-conditioned environments due to professional or personal reasons (Diette et al. 2007). Reports of headache, fatigue, irritation of eye, nose, throat, dizziness and nausea due to inadequate fresh air intake is not uncommon in closed air-conditioned (A/C) environments (Cho et al. 2006). The indoor air quality is influenced by the presence of abiotic as well as biotic agents like dust particles and microorganisms including fungi, bacteria, viruses, mites, etc. (Salo et al. 2006). The indoor air quality thus may be a public health issue, since the health of people who live or work in these environments is affected (Cooley et al. 1998; Mitchell et al. 2007). The microorganisms present may be allergenic, toxic or pathogenic. People who are immunocompromised could be at risk since they may be easily infected even by non-pathogenic organisms (Simon et al. 2008).

Fungi are cosmopolitan in distribution and are a serious threat to public health in indoor environments (Samet and Spengler 2003). Many fungal species that are reported to cause allergy belong to Ascomycotina, Basidiomycotina or anamorphic fungi. There are many reports on fungi isolated from indoor environments (Portnoy et al. 2005). It is important to know the diversity of organisms present and their effects so that public health strategies can be developed. In this study we have investigated the fungi from air-conditioned

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environments and the fungi causing allergies are identified.

2 Materials and methods

A survey of A/C rooms, such as computer laboratories, hospitals, CT scan centers, etc., was conducted in Nizamabad District of Andhra Pradesh State of India. A questionnaire was prepared and data were collected from people who live or work in air-conditioned rooms. Responses of the participants were analyzed to understand the practices that may contribute to the health of indoor environments. Particulars about the usage, cleaning of filters, room, etc., were collected.

2.1 Isolation and identification of fungi

Potato dextrose agar plates in triplicates containing 40 mg l^{-1} Rose Bengal were exposed to air from A/C units for 20 min. Sampling interval was once a fortnight for 3 months or weekly once for 2 months. Plates were incubated at 28°C for 5 days. After the completion of the incubation period, colonies of fungi were counted and identified. The fungi were identified based on their macroscopic and microscopic features (Raper and Fennell 1965; Domsch et al. 1980; Samson and Pitt 2000). Potato dextrose agar was purchased from Hi-Media Laboratories, MS, India.

2.2 Extraction of fungal allergic extracts

Fungal allergenic extract of 11 indoor fungal isolates and standard fungal isolates were made by Creative Drug Industries, Vashi, Mumbai, India, by the following procedure. Cultures of fungal isolates were grown in Erlenmeyer flasks in center mold medium at room temperature for 4 days and subcultured in fresh center mold medium to obtain maximum mycelial mass at room temperature for 3 weeks. 4 ml of 0.5% formalin was added to each flask, and kept for 24 h to kill the mycelial growth. The culture broth was decanted and the mycelial mass was kept at 50°C for 4 days, then 50 gm of mycelial mass was taken for extract preparation. Dried mass was mixed with Coca solution, followed by cold centrifugation at 4°C and ultrafiltration. Extract was diluted in phosphate buffered saline (1:50), the protein nitrogen unit (PNU) was estimated by Lowry's method (Lowry et al. 1951) and in vivo

toxicity assay was conducted. The extract was glycerinated and checked for specific gravity, phenol content, sodium chloride content, glycerol content, and finally PNU. Specific gravity, phenol content, sodium chloride content, glycerol content were checked as per Indian pharmacopoeia (Anon 2007).

2.3 Skin prick test for indoor fungal allergenic extract

Seventy-five patients were tested over a period of 3 months. All the subjects were suffering from atopic allergy viz. sneezing, rhinorea, nasal blockage, urticaria and asthma. These patients were referred by ENT specialists, chest physicians, and dermatologists for allergy testing. The preferred suitable area for skin prick test was on the back of the patient. The skin surface was cleaned with 70% alcohol then sites were marked using a ballpoint pen for negative control (saline), allergens of pollen, dust, dust mites, fungi (standard), and indoor fungal extracts (under study) and positive control (histamine). The test sites were $\sim 3 \text{ cm}$ apart. A drop of fungal allergenic extract solution of 11 indoor fungal isolates (under study), allergens of pollen, dust, dust mites, fungi (standard), negative control and positive control was applied separately to the skin next to the mark. The sterile lancet was then placed at an acute angle to the skin and a shallow lift was made. The lancet was raised for a second before the skin was released and this was repeated for each drop of solution. The lancet was wiped carefully on tissue of cotton wool, washed in sterile water and sterilized before using each solution. The excess solution on the skin was removed by blotting with tissue paper. The tests were allowed to incubate for 10–20 min. The results for positive reaction appeared as weals and erythema which were measured by using a scale in centimeters.

Skin prick tests were done in the clinic of Dr. Sunaina Waghay, Kamineni Hospital, Hyderabad, India.

3 Results

3.1 Common practices of the owners of air-conditioned rooms

Most of the A/Cs were switched on for a period ranging for 6–12 h. Half or more of the time/day A/Cs

were switched off. Not many users were cleaning the A/C, except at the time of repair or servicing. Filters inside the A/C were rarely replaced or cleaned. Very few of the owners used vacuum cleaners for cleaning their A/C rooms. Removable or periodically replaceable filters were not present in A/C units or owners were even not aware of the filters. The suppliers of the A/C units were not keen on appraising their customers about the importance of cleaning filters or A/C units.

3.2 Isolation and identification of fungi

Six genera of fungi like *Aspergillus*, *Penicillium*, *Neurospora*, *Fusarium*, *Trichoderma* and *Alternaria* were predominant in A/C rooms. Seven species of *Aspergillus* were identified, viz. *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus terreus*, *Aspergillus versicolor* and *Aspergillus parasiticus* (Table 1). Among the locations studied, computer lab III was noted with highest colony counts of up to 75 per plate. All the locations were contaminated with various *Aspergillus* species. *Neurospora crassa* was isolated from all hospital locations. *A. niger* and *A. fumigatus* were found in all the locations like computer laboratories and hospitals and were contaminating the majority of the places studied.

3.3 Allergenicity of fungi

Allergenic extracts made from the indoor fungal isolates (*A. niger*, *A. oryzae*, *A. fumigatus*, *A. terreus*, *A. nidulans*, *A. versicolor*, *A. parasiticus*, *Penicillium citrinum*, *Fusarium oxysporum*, *Trichoderma viride* and *Alternaria alternata*) were tested for their allergenicity by skin prick test on patients with control and standard allergenic fungal extracts. Extracts of only three fungal isolates were potentially allergic, i.e., *A. fumigatus*, *A. niger* and *Penicillium citrinum* (Table 2). *A. niger* was found in 80% of the locations selected in our study and *P. citrinum* was found in only one location. Computer lab I of our study was contaminated by five fungal species, out of which three species were found allergic.

4 Discussion

Results of this study indicate that the air quality of A/C rooms in the studied area may not be very good. Three allergenic fungi were found in nine locations out of the ten studied. *A. niger* was the most predominant fungus. The allergenic fungi were absent only from the hospital superintendent's room. The allergenic fungi

Table 1 Fungal flora and number of colonies/plate in air-conditioned rooms

Sample no.	Location	Samples collected	Frequency of sampling	Colonies (av. no.)	Fungi
1	Computer lab-I	08	Weekly once for 2 months	15	<i>Aspergillus fumigatus</i> , <i>A. niger</i> , <i>A. oryzae</i> , <i>Penicillium citrinum</i> , <i>Alternaria alternata</i>
2	Computer lab-II	08	Weekly once for 2 months	20	<i>A. fumigatus</i> , <i>A. niger</i> , <i>A. oryzae</i> , <i>Fusarium oxysporum</i> , <i>Trichoderma viride</i>
3	Computer lab-III	06	Fortnightly for 3 months	75	<i>A. fumigatus</i> , <i>A. niger</i> ,
4	Hospital labor room	06	Fortnightly for 3 months	12	<i>A. fumigatus</i> , <i>A. niger</i> , <i>Neurospora crassa</i>
5	Hospital scan room	06	Fortnightly for 3 months	04	<i>A. niger</i> , <i>A. parasiticus</i> , <i>N. crassa</i> , <i>F. oxysporum</i>
6	Hospital operation theater	06	Fortnightly for 3 months	08	<i>A. fumigatus</i> , <i>A. niger</i> , <i>N. crassa</i>
7	Hospital superintendant cabin	06	Fortnightly for 3 months	03	<i>A. terreus</i> , <i>N. crassa</i> , <i>A. alternata</i>
8	Nursing home operation theater	06	Fortnightly for 3 months	05	<i>A. fumigatus</i> , <i>A. niger</i> , <i>N. crassa</i>
9	Dental clinic surgery room	06	Fortnightly for 3 months	30	<i>A. fumigatus</i> , <i>A. nidulans</i> , <i>A. terreus</i> , <i>N. crassa</i>
10	CT scan center	06	Fortnightly for 3 months	04	<i>A. fumigatus</i> , <i>A. niger</i> , <i>A. versicolor</i> , <i>F. oxysporum</i>

Table 2 Allergenicity of fungi (antigenic extract) by skin prick test

Sample no.	Fungal antigenic extract	Allergenicity
1	<i>Aspergillus niger</i>	Allergic
2	<i>A. fumigatus</i>	Allergic
3	<i>A. terreus</i>	Non-allergic
4	<i>A. nidulans</i>	Non-allergic
5	<i>A. versicolor</i>	Non-allergic
6	<i>A. oryzae</i>	Non-allergic
7	<i>A. parasiticus</i>	Non-allergic
8	<i>Penicillium citrinum</i>	Allergic
9	<i>Fusarium oxysporum</i>	Non-allergic
10	<i>Alternaria alternata</i>	Non-allergic
11	<i>Trichoderma viride</i>	Non-allergic

could be a health threat to the employees/patients since they may breathe in the fungal propagules leading to allergic problems and other health hazards. The highest colony count was noted in computer lab III, while 80% of the locations harbored *A. fumigatus*, a potential agent of pulmonary aspergillosis and angioinvasive as well as opportunistic pathogen (de Hoog et al. 2000). Colonies of *A. fumigatus* were predominant in Computer lab I, II, III, and the hospital labor room, operation theaters of hospital and nursing home, and CT scan center. The fungal spores need relatively high velocities (~ 20 m/s) to attain high efficiency impaction, but the fungal spores present in the enclosed areas as in the present study also get deposited on agar plates though it may have certain limitations compared to high efficiency impaction.

Penicillium species are reported occasionally to cause infections in humans and the disease is known as penicillosis. It is acquired by inhalation and results in initial pulmonary infection, followed by fungemia. Most *Penicillium* infections are found in immunosuppressed hosts (Goh et al. 2007). *P. citrinum* is a fungus ubiquitous in the environment, and rarely reported to cause mycotic keratitis (Gugnani et al. 1978), urinary tract infections (Gilliam and Vest 1952), pneumonia with pericarditis (Mok et al. 1997) and hypersensitivity pneumonitis (Yoshikawa et al. 2005).

Fusarium species are reported as causative agents of superficial and systemic infections in humans referred to as fusariosis (Howard 2003). Opportunistic infections develop in immunosuppressed hosts,

particularly in neutropenic and transplant patients (Guarro and Gene 1995). The presence of *Fusarium* in hospital water systems may result in fusariosis in immunosuppressed patients. *F. oxysporum* may also exist in the soil of potted plants in hospitals and serve as mycotic reservoir for nosocomial fusariosis (Ortoneda et al. 2004). *Fusarium* species produce mycotoxins such as Fumonisin and Zearalenones (Austen et al. 2001).

Alternaria species isolated as indoor inhabitants of A/C rooms produce nasal and subcutaneous lesions, and nail infections; the majority of infections being reported from persons taking immunosuppressive drugs. They produce the antifungal Alternariol and metabolites such as AME (alternariol mono methyl ether), tenuazonic acid, and altertoxins (mutagenic; Vennwald et al. 1999). Airborne *A. alternata* conidia were found in both indoor and outdoor environments of numerous studies, resulting in allergic rhinitis and asthma (Green et al. 2005; Seitz et al. 2008).

Trichoderma species cause human infections like peritonitis in dialysis patients and perihepatic infection in liver transplant patients. They are emerging opportunists in immunocompromised persons and produce trichothecene and cyclic peptides, gliotoxin, isocyanides, T-2 toxin, and trichodermin. *Trichoderma* may cause mycotoxicosis similar to that caused by *Stachybotrys chartarum* (Groll and Walsh 2001). *T. viride* has been reported as a causative agent of pulmonary fungus ball in continuous ambulatory peritoneal dialysis (CAPD) and associated peritonitis (Kwon-Chung and Bennett 1992; Bren 1998).

Our survey revealed some interesting facts about the users of A/C rooms with the following practices that may be contributing to the indoor air quality. Most of the A/Cs were shut off for 6–12 h per day. This may add to relative humidity and may favor fungal growth. A/C filters were rarely cleaned, thus they may act as reservoirs of fungi. There may also be source material such as used papers, used cotton plugs, etc. Good sanitary practices like frequent changing of the A/C filters, cleaning of the rooms, and keeping the A/C units switched on for 24 h, may help in reducing fungal contamination. Knowledge of the microbial diversity of A/C rooms may help in taking remedial measures to reduce their incidence.

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