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Assessment of Airborne Fungi in Children's Hospital Located in Kolkata (India)

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ABSTRACT

Fungal spores contribute significant concentration in the bioaerosol of various environmental conditions which may have potential health threats. Our study was aimed at determining the presence of disseminating airborne fungi in a pediatric government hospital in Kolkata. The study was started from the post-monsoon to the middle winter (August to December 2008) in the indoor and outdoor environment of the hospital with temperature and humidity ranges of 11.2 °C-35.2 °C and 70 per cent-90 per cent, respectively. Air sampling was performed at 14 days intervals during the daytime following the gravitation settling method, and the fungal colonies were identified based on micro and macro morphological characteristics. The percentage contribution of individual fungal species from the outdoor section and indoor units (Newborn Baby Ward, Respiratory Care Unit, Step Down Ward, Thalassemia Care Unit) of the hospital environment was calculated. We observed profound aeromycofloral diversity where the outdoor environment was mostly colonised by sterile hyphae (16.43 %) along with the allergenic *Aspergillus fumigatus* (13.6 %) and *Penicillium sp.* (12.32 %). Conversely, an abundance of *Cladosporium herbarum* (24.7 %) and *Penicillium sp.* (17.85 %) followed by *Aspergillus sp.* (12.9 %) and sterile hyphae (14.51 %) were found in different indoor units. Our results showed the diversity of airborne mycoflora which promotes the trend to health difficulties and thus the hospital environment monitoring along with proper control measures is essential.

Keywords: Airborne fungi; Hospital environment; Fungal diversity; Monitoring

1. INTRODUCTION

Aeromycoflora denotes the composition of the airborne fungal spores in a given area. Both beneficial and harmful fungal species are present ubiquitously in various environmental conditions. Hence, study of fungal diversity in an environment is essential to determine the possible applications of these fungal species in the pharmaceutical and food industries. On the other hand, periodic assessment of fungal diversity in the environment would be of great significance to manage the emerging health threats caused by some fungal pathogens. Therefore, the aeromycoflora in hospital environment may have great epidemiological concern and cause significant economic loss in terms of human mortality and morbidity[1-2]. The indoor and outdoor units of hospitals, health care units, and wellness centers should have proper measures to have naught or least fungal count to abolish invasive fungal diseases. However, when it comes to assessing the air samples in our daily lives outlines, there is essentially no fungus-free background[3-4]. Starting with this key problem, the study of aeromycoflora diversity in different environmental conditions should be carried out very promptly. In many cases, airborne fungal species have deleterious effects i.e. a large percentage of them are significant contributors to adverse health effects. They cause a threat to the human health and wellbeing of the population with the potential source of some health hazard materials such as mycotoxins. These types of fungal spores are already making significant contributions to plant pathology, human respiratory disorders and other undesirable effects such as infections, hypersensitivity pneumonitis, and toxic reactions[5-10]. With great access to indoor and outdoor environments, fungi restore allergy to almost 10 per cent of the global population[11].

Indoor Air Quality (IAQ) is a crucial factor in hospital facilities for preventing hospital-associated infections, sick hospital syndrome, and a range of occupational risks[12]. Research investigations have revealed that several hospital infections were caused by fungi, such as *Candida albicans*, *Aspergillus sp.*, *Cladosporium sp.*, and *Penicillium sp.*[13-16]. Various investigations continued to monitor the diversity of mycoflora composition and bioaerosol characteristics of the hospitals worldwide to determine the possible health risks of this airborne mycoflora[17-20].

Therefore, frequent air quality investigation and monitoring with the ambitious vision for solving the problem are required. With the particular concern to the hospital environments, assessing the quality of air is intrinsically required as patients

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may have a great extent of additional toughness to serve as a source or prone to pathogenic microorganisms. Our study aimed to determine airborne fungal diversity in the typical hospital environment in the 'City of Joy' Kolkata. Here, we investigated the concentration of airborne fungi in the pediatric government hospital in eastern Indian state West Bengal. The aeromycoflora in this children's hospital environment could produce a vital threat to child health and may lead to fatal incidents. Hence, regular assessment of the diversity of airborne fungal spores in the hospital is necessary to determine the mycofloral concentration and associated health hazards and epidemiological circumstances. The purpose of the investigation was to understand the fungal flora associated with air quality and its influence on population-related to hospital vicinity so that the masking problem may take into solution accountability.

2. MATERIALS AND METHODS

2.1 Sampling Site

The indoor and outdoor wards of a renowned and busy government children hospital drew our attention as the sampling site for the current aerobiological study. It is one of the renowned and busy government children hospitals in Kolkata. Starting from newborns, children admitted to this hospital sometimes along with their mothers or other family members in crisis. However, the hospital is always entrusted with the task of providing holistic health care but being ubiquitous in nature fungi grow continuously by virtue in this environment. However, to date, no scientific report is available on the microflora studies of this hospital environment. The various places of investigation, therefore, may have a significant health effect as the production, concentration, and contamination of fungal pathogens are correlated with different natural parameters like air quality, moisture content, relative humidity, and temperature, etc. with the seasonal and environmental variations.

2.2 Media Preparation

An appropriate culture medium supporting nutritional needs induces the production of fungi. Several growth media are available which are very much suitable for extensive fungal growth. For this assay, Malt Extract Agar (MEA) media, containing malt extract 20 gmL-1 and agar 20 gmL-1 of distilled water has been used and prepared aseptically. Then the liquid media was poured into sterile Petri dishes following aseptic techniques. Once the media solidified, the edges of the Petri dishes were sealed using sellotape. The cool Petri dishes were covered with brown paper and taken to the sampling site.

2.3 Sampling procedure in the hospital environment

The study was conducted between August and December 2008. The Petri dishes were taken to the study site to trap the fungal composition. The sampling sites were broadly divided into the indoor environment [comprising of four sampling sites such as New Born Baby Ward (first floor of the oldest building block), Respiratory Care Unit (most recent building-annex building), step down ward (second floor of the oldest building block), and Thalassemia Care Unit (situated in the oldest building block)] and outdoor environment of the hospital building.

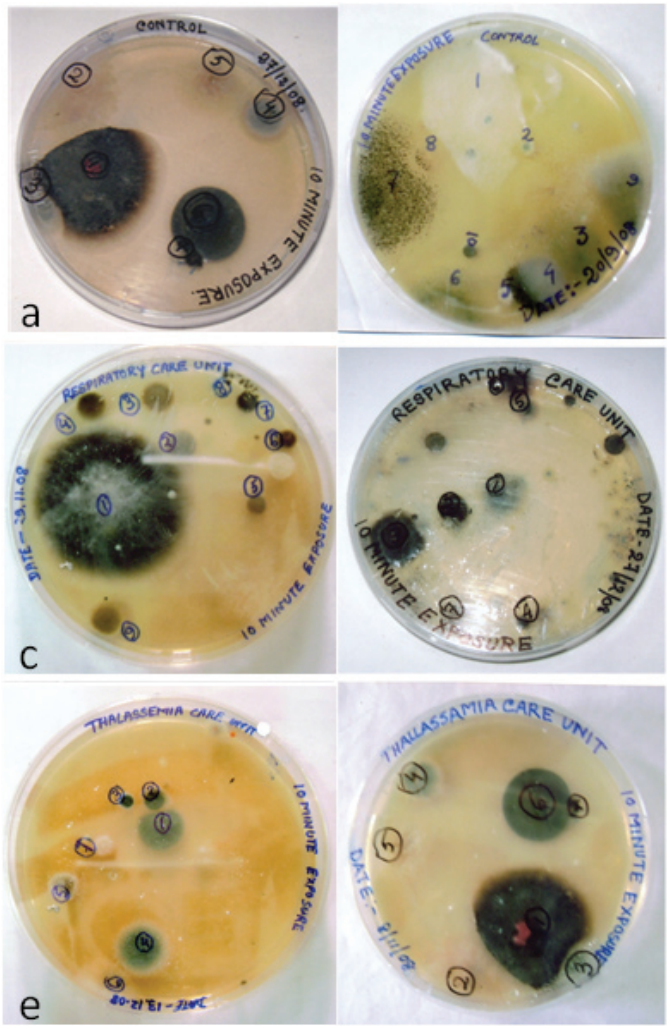
Air sampling was performed at the height of about 1.5 m - 2 m above the ground of the investigation site during day-time (12 pm to 2 pm) at 14 days intervals. One Petri dish, used for each sampling in five investigation sites was done on a visiting day. The Petri plate Gravitational method was used for the isolation of fungi[21-24]. Petri dishes were exposed to the air for 10 min and then covered by the lid and again sealed by sellotape. Then the plates were taken into the laboratory within 2 hr and incubated for 3-5 days at room temp (25 ºC-28 ºC). The seasonal and meteorological variations had an intense effect on the incidence of airborne fungal species both qualitatively and quantitatively[25-26]. So, meteorological data were collected from India Meteorological Department, Regional Meteorological Centre, Alipore, Kolkata and recorded on each sampling date (Table 1).

Table 1. Temperature and humidity on different sampling dates

Date	Temperature (°C)		Average humidity (%)
	Maximum	Minimum	
16.08.08	32.2	17.1	90
06.09.08	35.1	24.2	84
20.09.08	35.2	25.2	84
04.10.08	32.0	24.0	87
18.10.08	33.4	23.8	70
08.11.08	32.4	20.6	70
29.11.08	28.5	16.8	73
13.12.08	31.0	17.0	75
27.12.08	24.5	11.2	80

2.4 Identification and Characterisation of Fungal Strains

The identification of fungal colonies was made by visual and microscopic examinations. Visual emergence of fungal colonies arises on the 3rd and 4th days, from the incubation (at 21 °C–28 °C) and was associated with characteristic features. The fungal colonies were counted based on their gross colonial appearance i.e. macro-morphological properties. Compound microscope was used to determine the colonial feature and morphological structure of fungi. Fungal morphology was determined by mounting the material in lacto phenol and cotton blue. Fungal species were identified based on macro morphology (reverse and surface colouration of the colonies, characters of the colony i.e. effusive or not, cottony or dusty etc.), micro morphology of the organisms (hyphal colour, spore colour, conidial colour, hyphal breadth, spore length and breadth, number of cells in each spore, spore shape and attachment of the spore with conidia etc.) grown on MEA media. Identification of fungi was carried out up to the genus level and



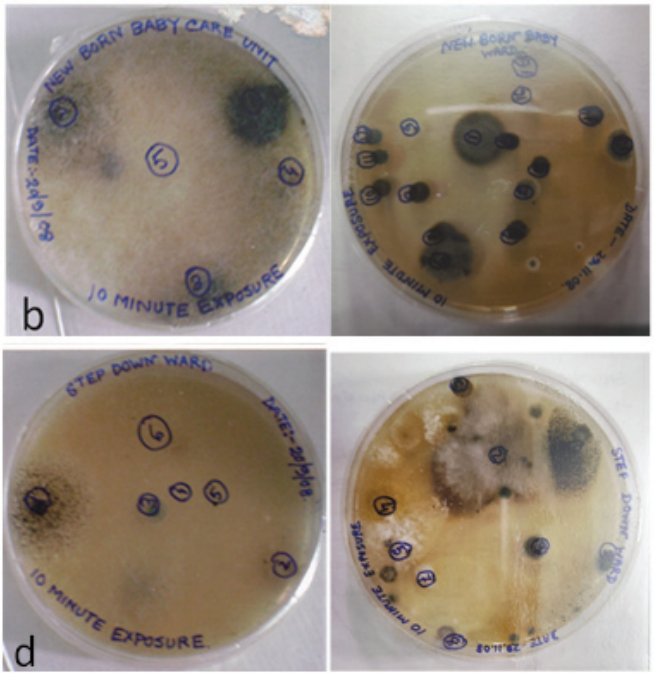


Figure 1. Visual emergence of fungal colonies in Petri dishes with characteristic features from a) Outdoor Unit, b) Newborn Baby Ward, c) Respiratory Care Unit, d) Step Down Ward, and e) Thalassemia Care Unit of the children's hospital, Kolkata.

in some cases up to species level as described previously[27-28]. Then a study was accompanied by an analysis of percentage contributions of individual fungal species to search fungal spore concentration and it was calculated as per the formula: % Contribution = (Total number of colonies of one species / Number of colonies of all species) × 100

3. RESULTS

The data received from different units of the hospital considered diverse information and highly relevant records. This four months aeromycofloral study revealed the ubiquitous distribution of fungi in the indoor and outdoor environment of the hospital. Our aerobiological sampling data indicated the high occurrence of various fungal spores in the hospital (Fig. 1).

The occurrence of airborne fungi varied each day depending on the natural parameters like moisture content, temperature, humidity, average rainfall, etc. The scheming parameters in the air and accumulate a different range of fungal spores. In the Outdoor Unit, the count (%) of fungal colonies showed the prevalence of *Aspergillus fumigatus* (14 %), *Penicillium sp.* (13 %), *A. niger* (11 %), *Cladosporium sp.* (11 %), *Aspergillus sp.* (6 %), *A. flavus* (4 %), *Alternaria sp.* (4 %), and *Corynespora cassicola* (3 %), etc (Fig. 2a). The indoor environment comprises of four different sites: Newborn Baby Ward, Respiratory Care Unit, Step Down Ward, and Thalassemia Care Unit. The higher abundance of *Cladosporium herbarum* (24 %), *Penicillium sp.* (18 %), *Aspergillus sp.* (13 %), and sterile hyphae (14 %) were found in the different sections of the indoor environment (Fig. 2b-e). In New Borne Baby Ward, *Cladosporium herbarum* (24 %) showed the highest occurrence followed by *Penicillium sp.* (14 %), *Aspergillus fumigatus* (10 %), *A. niger* (9 %), *Corynespora cassiicola* (5 %), etc. In the Respiratory Care Unit, fungal colony (%) showed the prevalence of *Penicillium sp.* (18 %), *Cladosporium herbarum* (16 %), *Aspergillus niger* (14 %), *A. flavus* (5 %), *A. fumigatus* (4 %), *Fusarium sp.* (4 %), etc. In step down ward, occurrence of fungal colonies was dominated by *Penicillium sp.* (13 %), *Cladosporium herbarum* (8 %), *Aspergillus fumigatus* (6 %), and *A. niger* (3 %), etc. In Thalassemia Care Unit, *Penicillium sp.* (13 %), *Cladosporium sp.* (10 %), *Corynespora sp.* (5 %), *Aspergillus niger* (3 %), and *Fusarium sp.* (3 %) showed higher occurrence (Fig. 2, Fig. 3).

4. DISCUSSION

Hospital is a social need to provide safe, clean, and beneficiary treatment from small to large scale for going miles ahead in the journey of life. For this reality, hospitals and other healthcare centers require credible testing of air quality, and effective ventilation should be allowed for passing out the hazardous emissions with the target of patients accompanying comfort[29-30]. Our present study was aimed at the assessment of aeromycoflora in the children's hospital, Kolkata for the detection of fungal contributors and amplifiers. Fungal

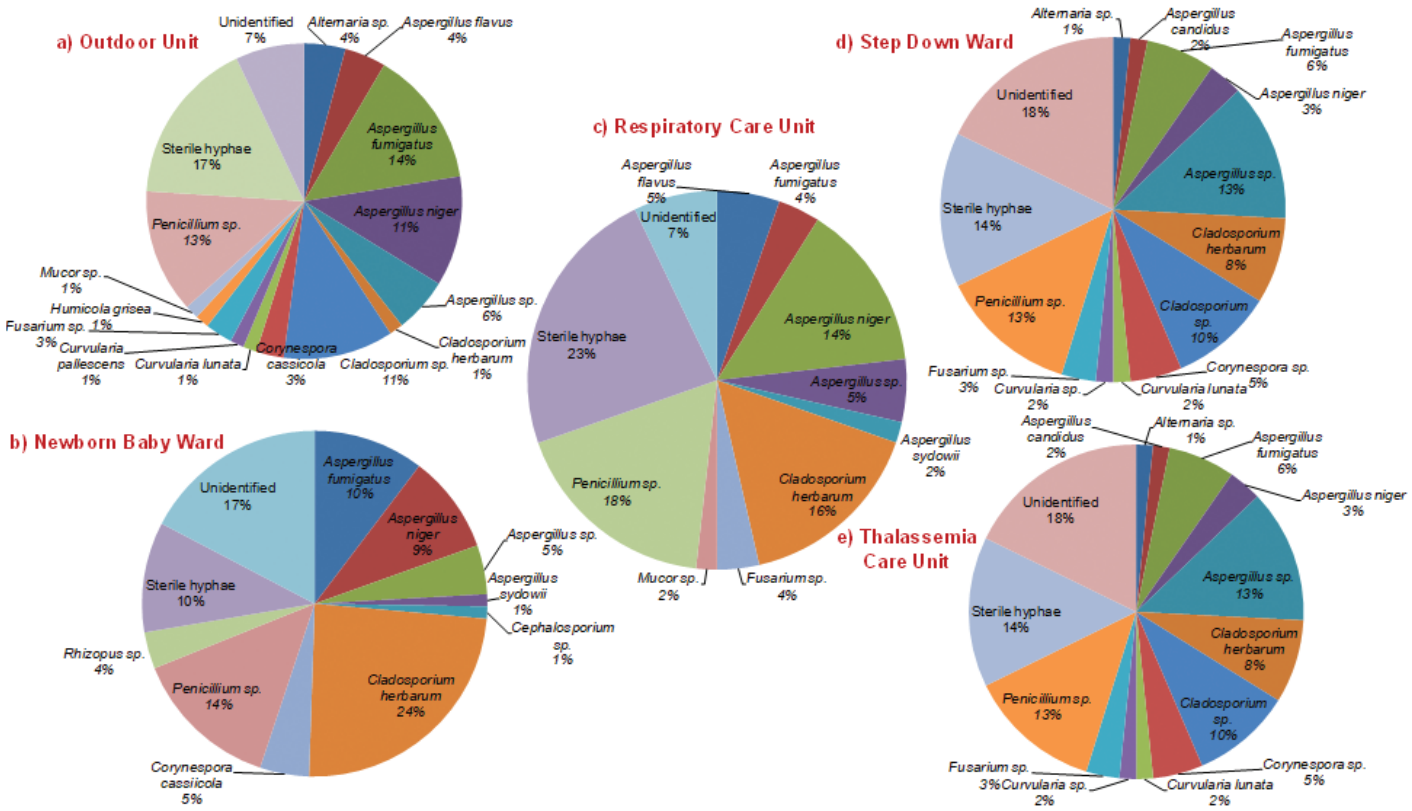


Figure 2. Relative distributions of fungal colonies from a) Outdoor Unit, b) Newborn Baby Ward, c) Respiratory Care Unit, d) Step Down Ward, and e) Thalassemia Care Unit of the children's hospital, Kolkata.

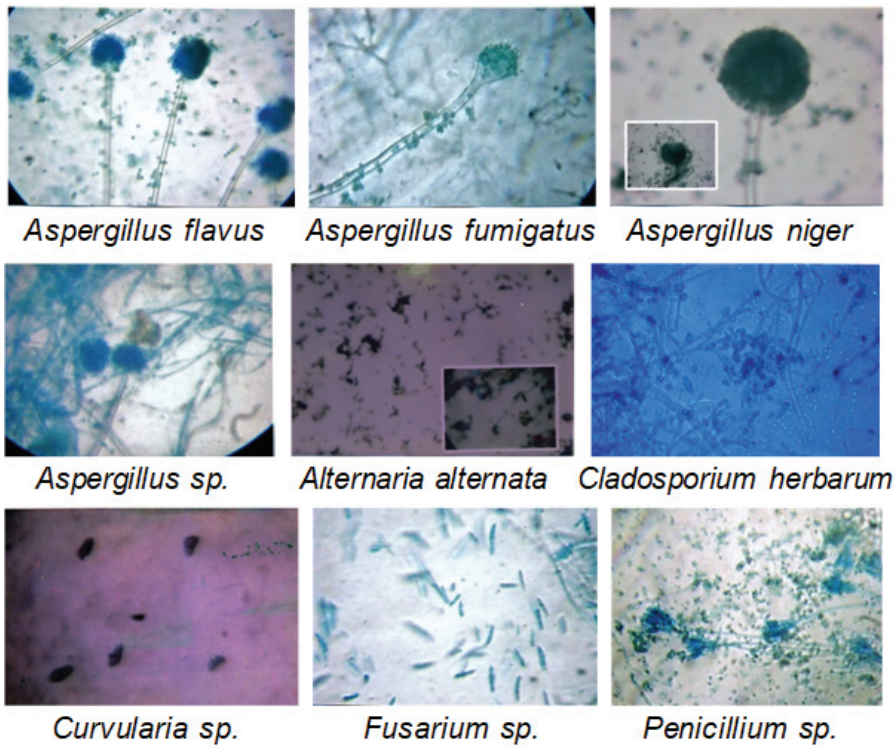


Figure 3. Abundant fungal spores from indoor and outdoor environment of the children's hospital, Kolkata under compound microscope (100X).

distribution and propagation correlate with the environmental changes which fluctuate over the meteorological condition and seasonal variation. Therefore, our investigation was essential to safeguard this important child hospital from the harmful aeromycoflora which could be helpful to measure the future standards for social needs.

In our investigation, the prevalent fungi isolated from the indoor air of the four different wards of the hospital were of the genus *Aspergillus, Cladosporium,* and *Penicillium.* The most abundant species of *Aspergillus* were *A. flavus, A. fumigatus, A. niger,* and *A.* sedowii, whereas, *Cladosporium* was represented by the species like *C. cladosporoides, C. herbarum,* and *C. oxysporium* (Fig. 2b-2e). So, it was evident that the fungal population recorded from the hospital showed a similar pattern with the previous studies by other researchers towards the monitoring of airborne fungi in the hospitals from different parts of the world. Our data was collected over time (from August to December) and there was a difference in fungal composition with time. In the outdoor unit of the hospital, the following

fungal species *viz. Cladosporium sp, Aspergillus flavus*, and *Aspergillus niger* were prevalent in August, September, and October, respectively. *Aspergillus fumigatus* was dominant in November and December in the outdoor unit. In the new borne ward, *Aspergillus fumigatus, Cephalosporium sp., Corynespora cassiicola* were dominant in August. *Aspergillus niger* was found as the dominant fungi in October in the new borne ward. *Cladosporium herbarum* was found to be prevalent in September, November, and December in new borne ward. In September and October, *Cladosporium herbarum* and *Penicillium sp* were prevalent in the respiratory care unit. *Aspergillus sedowii, Aspergillus sulphureus,* and *Cladosporium sp* were found dominant in August, November, and December, respectively in the respiratory care unit. In step down ward, *Cladosporium herbarum* was dominant in August, November, and December. *Aspergillus niger* and *Penicillium sp* were found prevalent in September and October in step down ward. In the thalassemia care unit, *Alternaria sp., Aspergillus sp., Cladosporium sp, Curvularia lunata,* and *Penicillium sp.* were prevalent in August. In September, *Cladosporium herbarum* was the dominant species. *Aspergillus sp., Penicillium sp.* and *Aspergillus fumigatus* were prevalent in October, November, and December in thalassemia care unit. Overall, *Cladosporium herbarum* was prevalent in August and September in the hospital environments. In October, *Aspergillus niger* and *Penicillium sp* were found dominant. *Aspergillus fumigatus* and *Cladosporium herbarum* were prevalent in November and December, respectively, in the hospital environments.

Several investigations command this fact and even instill. The emergence of airborne microflora in hospital rooms was the subject of various surveys and studies as a promising cause of hospital infections[17-20]. All the critical analysis of the hospital survey apprised that the environment was associated with different types of fungal spores. Air samples collected by the Petri plate gravitational method showed fungal contaminants in both outdoor and indoor environments. The genus *Penicillium* exhibited the greatest spectrum in the hospital environment.

Contamination of outdoor air is typically caused by pathogenic fungi like *Aspergillus* which is not only allergenic but they can produce a number of health hazard materials such as mycotoxins[31-33]. The dominant colonies of *Aspergillus* were represented by different species namely *A. flavus, A. fumigatus,* and *A. niger.* Air plays a key role in the dissemination of *Aspergillus* and therefore transmission to the susceptible[34]. *Aspergillus* conidia even may remain in the air for a long duration. Due to substantial concentrations of *Aspergillus* they revealed a large number of risk factors like aspergillosis and other kinds of severe allergies.

The observed fungal counts confirmed the presence of fungal spores in the indoor and outdoor environment of the hospital. A higher percentage contribution of *Aspergillus spp.* in both types of environment is the hazardous sources of pathogenic fungi. The viability of *Aspergillus sp.* in the air for a prolonged time indicates increased health hazards. The data in our study revealed a similar relationship in the indoor and outdoor environment of the hospital where available *Aspergillus spp.* has a great sensitivity to the environment. The genus *Aspergillus* is very often involved in the incidence of aspergillosis, ear, and skin infections[35], and in most cases, *Aspergillus* infection occurs via inhalation. So, appropriate action should be taken for the safe removal or any accumulations repeat of *Aspergillus spp*. Besides, the concentration of *Penicillium spp.* population in different wards of the hospital along with the outdoor environment was also recorded. The genus *Penicillium* is generally acquired by inhalation and results in initial pulmonary infection, followed by fungemia. Other fungal genera obtained in this study were *Alternaria, Cladosporium, Curvularia, Fusarium, Humicola, Mucor*, etc. These all are common genera frequently isolated from indoor environments of hospital and air conditioning systems. Even though these are apparently harmless for healthy people, it may be unsafe for the patients of risk groups (immuno-suppressive, immune-compromised, and immune-competent), especially those treated in surgical wards, newborn ward, and intensive care units[36-37]. Other species that had been isolated in our case study with low concentration reported having antagonistic health symptom[38]. The role of infections caused by mould *Mucor* and *Rhizopus* generally occurs in severely ill patients and may lead to life-threatening mucormycosis.

5. CONCLUSION

This is the first report that revealed the prominent occurrence of various fungal species belonging to the genera *Aspergillus, Penicillium, Alternaria, Cladosporium, Curvularia, Fusarium, Humicola, Mucor*, etc. from the different outdoor and indoor areas of the hospital. Combining with the meteorological parameters like temperature, humidity, relative rainfall occurrence of fungal spores showed different magnitude. This viewpoint reminds us that the dominant population of aeromycoflora is also related to seasonal and altitudinal variations. Our findings could be explained by various factors including human activities. The result could be attributed to the high rate of in and out the movement of people. The materials brought from outside by the visitors seem to be the potential direct or indirect sources of mycofloral contamination. Thus, the environment where patients are treated has an important influence on the likelihood of such recovering or acquiring an infection that may complicate their conditions. So the fungal flora of the hospital should be determined and controlled routinely. This investigation revealed the generating fungal diversity which promotes the trend to health difficulties and thus the aerobiological monitoring of the hospital environment along with proper antifungal control measures is strongly required. As a precautionary measure, cleaning operations and maintenance actions of the various outdoor and indoor areas of the hospital should conducted on a regular basis. Further studies are also required to understand the molecular diversity, volumetric analysis of the aeromycoflora, and their allergic properties in the hospital environment.

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