A preliminary assessment of Aerofungal Allergens from the wards of civil hospital Aizawl

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ABSTRACT

Air is one of the most important components of environment as well as for living creatures. The air we inhale is heavily infested with a variety of biological particles in addition to inorganic substances such as gas, dust and smoke. Bio-aerosols are air borne particles that are living (bacteria, viruses and fungi). The health effects of bio-aerosols including infectious diseases, acute toxic effects, allergies and these coupled with the threat of bioterrorism and SARS have led to increased awareness on the importance of bio-aerosols. The evaluation of bio-aerosols includes use of variety of methods for sampling depending on the concentration of microorganisms expected. Fungal allergens can cause various health defects especially in sensitive hosts, depending on the kind of mycotoxin and the nature of the exposure effects includes mucous membrane irritation, skin rashes, dizziness, nausea, immunosuppression, birth defects, cancer, Sick Building Syndrome (SBS) and Building Related Illness (BRI) which are frequently reported, the symptoms include eye irritation, skin irritation, head ache, drowsiness, reduced mental capacity, mental fatigue etc. The hospital environment can also cause serious problems to the patient whose immunity is already low. So it is important to the hospitals to identify and assess the quantity of the indoor mycoflora and hence the current project carried out in Civil Hospital Aizawl, (CHA) Aizawl, Mizoram.

Totally 13 fungi were isolated from CHA, among them Aspergillus sp. were predominant and followed by Penicillium, Candida, Alternaria, Fusarium, Geotrichum, Curvularia, Cladosporium, Drechslera species etc. Present study has clearly demonstrated that the concentration of airborne fungi was high in CHA.

Keywords: Aizawl; Bio-aerosols; Deuteromycotina; Fungal allergens; Indoor molds; Mycotoxins.

1. Introduction

Air is one of the most important components of environment as well as basic needs of all living organisms. Most of the non pathogenic fungi and rare pathogens which contaminate the air are able to survive even the adverse condition of the outdoor air to cause diseases to human beings. Spores and fragments of moulds are numerous in the air. During coughing, sneezing and talking, varying numbers of droplets are expelled from the body which contains significant number of microorganisms.

Apart from the above Microbial Volatile Organic Compounds (MVOC) are also produced by fungi which are found to affect the occupant’s health. Amongst the different classes of
microorganisms Fungi and Bacteria have been found to be the most frequent risk factors of diseases and other health hazards in the environment. (Padma srikanth et al., 2008). Fungi produce large amount of spores which is easily become airborne, thus giving rise to an important microbial aerosols.

From the medical point of view any detail on the exposure of a population to fungal propagules are of importance in treating the subjects reporting fungal infections/allergy. (Rogers, 2003). Hence indoor air quality is becoming an important public issue. Inhalation of certain types of air borne spores and their metabolites within the building is widely accepted as one of the important causes of some respiratory disorders. (Splenger et al., 1983).

Airborne fungi causing respiratory infections and allergic reactions include Penicillium, Aspergillus, Acremonium, Paecilomyces, Mucor and Cladosporium (Lidwell, 1981). Most common has been Aspergillosis mainly occurs in immunocompromised hosts or as a secondary infection following inhalation of fungal spores or the toxins produced by them. Symptoms include persistent cold, watering eyes, prolonged muscle cramps and joint pain, etc (Hollaren. 1991).

Bio-aerosols, of which fungal spores are one of the major types of microorganisms, can be present in all hospital environments and may be transmitted through air, outdoor air, visitors, patients and air conditions( Beggs.,2003 and Lugauskas.,2004). The evaluation of microorganism count, types and diversity in hospitals rooms, especially in sensitive units like intensive care units (ICU) and surgery operation theatres (OT) has raised worldwide concern. Approximately all patient infections are suspected to be hospital- acquired (Manuel et al., 1998).These infections can have serious consequences in terms of increased mortality, morbidity and length of hospital stay and overall costs. (Rajkumar et al., 1999).

Hospital air contains a diverse range of microbial population. The significance of these microbes is debatable in some quarters, whereas elsewhere it may be considered significant. The importance of the estimation of the quantity and types of airborne microorganisms are that these values can be used as an index for the cleanliness of the environment as well as an index they bear in relation to human health and as source of Hospital-acquired infections. (Horner, 1995). The source and spread of organisms inside the hospital are important issues. Human related organisms or the body normal flora, also found in clothing are spread through shedding during human activities. (Ayliffe et al., 1999).

Geographical and regional monitoring of indoor microorganisms has not been extensively investigated. The present study was conducted to gain knowledge of the quantity and quality of airborne microorganisms in the wards of Civil Hospital Aizawl, and to set minimum acceptable standard of tolerable levels of microbial population.

2. Materials and methods

2.1 Study area

Mizoram was recognized as Lushai Hills and formed part of British India in 1898.Aizawl is the largest city as well as the capital of the state of Mizoram, India. It is located north of the tropic of Cancer in the northern part of Mizoram. It is situated on a ridge 1132 meters (3175 ft) above sea level, with the Tlawng river valley to its west and the Tuirial river valley to its east. It had a population of 228,280 (2001). Aizawl has a mild, sub-tropical climate due to its location and elevation. In the summer the temperature ranges from 20° C to 30° Celsius, and
in the winter 07º C-21º Celsius. The average rainfall for the last 20 years (1986 -2005) accounts to 2793.63 mm, with the highest precipitation recorded during April to October. The average relative humidity is 73.14%, with the highest percent is recorded during June to August. (Figure 1).

![Figure 1: Monthly average Rainfall, Relative Humidity and Temperature recorded during 2010 from Aizawl.](image)

The study was carried out at Civil Hospital Aizawl, Mizoram during the month of March to December 2010. The study was conducted in 6 different wards which includes Casualty(CAS), General OT(GOT), Pediatric ward(PED), Male Medical Ward (MMW), Female wards (FMW) and Intensive Care Unit (ICU). A total of 38 air samplings were performed in the internal atmosphere of all the wards.

2.2 Air sampling

Air sampling has been done using Himedia’s air sampling system (Himedia Laboratories Pvt.Ltd, Mumbai, India) for the isolation of cultivable molds. The device can hold an agar strip which contains Saubourad Dextrose Agar (SDA). The sampler was set at an air-sampling rate of 40 L/min for 5 minutes per sample. Samples were collected from the centre of the room away from open windows and doors, and the sampling position was 1 m above the floor. Each ward represented by large rooms. Each room has accommodated many patients without any partition. Sampling was done in all the operation rooms when there were no surgical procedures taking place. Every time the samples were collected during 10 am to 11 am in the morning and 2 pm to 3 pm in the evening. A total of 30 samples (one sample = one day collection) were collected during the study period that from March to December 2010.

2.3 Sample processing

After the air borne microbial samples were done on SDA (HiMedia, India), they were transported to laboratory and immediately incubated at 30ºC with daily observation of the plates for fungal growth upto 21 days. Counts of different fungal growth were coded as they appeared on SDA and were designated to their specific genus and species.
2.4 Fungal identification

After the collection is done the agar strips were transferred to their cover case which was then incubated at 30º C for minimum 7 days to maximum 21 days. The total number of colony forming units (CFU) were enumerated and converted to organisms per cubic meter in air. During incubation, yeast and filamentous fungi growing on SDA agar strips was sub-cultured on two separate SDA culture plates, incubated at 37ºC and 30ºC for 48 hours and 21 days respectively, and then identified based on the microscopic colony morphology and spore types. The fungal types were enumerated, identified and confirmed by using Standard textbooks and monographs. (Gillman., 1957; Udayaprakash., 2004) based mainly on colonial morphology, microscopic examination of the spore, and hyphal characteristics by lactophenol cotton blue preparations. And slide culture preparations were made to determine the nature of the fungal hyphae and the fructifications, such as conidiophores, conidia production. And then kind of conidia produced by the fungi were loosely examined.

Various standard methods were used to identify yeast colonies (e.g. Germ tube test for Candida albicans). Most of the fungal colonies were identified unto genus level but some are fully identified by species. The total number of colony forming units (CFU’s) was enumerated and converted to organisms per cubic meter air. The results are represented in % contribution and Isolation frequency by using Standard method.

3. Statistical analysis

Data obtained as the number of colony forming units (CFU) per plate from aerobiological sampling were converted to the number of colony forming units (CFU) per cubic meter of air for the slit sampler of Himedia, India which was used for the collection of air sample. Analysis of variance for all the data were performed using a Statistical Package for the Social Sciences (SPSS) version 17.0 was used for statistical analysis. Means were separated by the least significant differences (LSD) at α = 0.05.

3.1 Isolation frequency

The term isolation frequency is used to denote the number of sampling in which a fungus was recorded as against the total number of samplings, i.e., 6 different areas.

- Common = Present in 5 and more samples
- Frequent = Present in 3 to 4 samples
- Occasional = Present in 2 to 3 samples
- Sporadic = Present in 0 to 1 samples

3.2 Percent contribution

The term percent contribution refers to the contribution of individual species to the total and is calculated as follows.

Percent contribution = \( \frac{\text{No. of CFU / m}^3 \text{ of individual species}}{\text{Total no. of CFU / m}^3 \text{ of all the species}} \times 100 \) (1)
4. Results and discussion

From March to December 2010, a total of 30 air samplings were made from the atmosphere of different medical wards of Civil Hospital, Aizawl, Mizoram, India. (Table 1). A total of 3044 fungal colonies were isolated and recognized as 621 colonies (20.40%) appeared during March to May, 1350 colonies (44.35%) and 1073 colonies (35.25%) have appeared in May-August and September to December respectively. (Table 1). Molds comprised 82.42 % and yeasts comprised of 17.58%.

Table 1: Percentage contribution and isolation frequency of fungi colonies / m$^3$ isolated during three different Seasonal air sampling from medical wards of Aizawl Civil Hospital.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Name of the Fungi</th>
<th>Set I (March-May)&amp; % contribution</th>
<th>Set II (June-August) &amp; % contribution</th>
<th>Set III (September-December)&amp; % Contribution</th>
<th>Total</th>
<th>Isolation Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aspergillus sp</td>
<td>2.43</td>
<td>4.00</td>
<td>3.90</td>
<td>10.34</td>
<td>Common</td>
</tr>
<tr>
<td>2.</td>
<td>Alternaria sp</td>
<td>1.37</td>
<td>3.67</td>
<td>3.54</td>
<td>8.60</td>
<td>Common</td>
</tr>
<tr>
<td>3.</td>
<td>Candida sp.</td>
<td>1.05</td>
<td>3.87</td>
<td>3.61</td>
<td>8.54</td>
<td>Common</td>
</tr>
<tr>
<td>4.</td>
<td>Cladosporium sp</td>
<td>0.95</td>
<td>3.54</td>
<td>3.25</td>
<td>7.75</td>
<td>Frequent</td>
</tr>
<tr>
<td>5.</td>
<td>Curvularia Sp</td>
<td>2.23</td>
<td>3.77</td>
<td>3.18</td>
<td>9.19</td>
<td>Common</td>
</tr>
<tr>
<td>6.</td>
<td>Drechslera sp</td>
<td>1.74</td>
<td>3.74</td>
<td>2.43</td>
<td>7.91</td>
<td>Frequent</td>
</tr>
<tr>
<td>7.</td>
<td>Fusarium sp</td>
<td>1.80</td>
<td>3.77</td>
<td>3.38</td>
<td>8.96</td>
<td>Common</td>
</tr>
<tr>
<td>8.</td>
<td>Geotrichium sp</td>
<td>1.97</td>
<td>3.72</td>
<td>3.35</td>
<td>9.03</td>
<td>Common</td>
</tr>
<tr>
<td>9.</td>
<td>Gliocladium sp</td>
<td>1.11</td>
<td>1.73</td>
<td>0.94</td>
<td>3.74</td>
<td>Sporadic</td>
</tr>
<tr>
<td>10.</td>
<td>Penicillium sp</td>
<td>2.59</td>
<td>3.87</td>
<td>3.64</td>
<td>10.11</td>
<td>Common</td>
</tr>
<tr>
<td>11.</td>
<td>Scolecobasidium sp</td>
<td>1.07</td>
<td>3.73</td>
<td>1.34</td>
<td>6.07</td>
<td>Occasional</td>
</tr>
<tr>
<td>12.</td>
<td>Scopulariopsis sp</td>
<td>0.85</td>
<td>1.36</td>
<td>2.10</td>
<td>4.27</td>
<td>Occasional</td>
</tr>
<tr>
<td>13.</td>
<td>Torula sp</td>
<td>1.24</td>
<td>3.58</td>
<td>0.59</td>
<td>5.42</td>
<td>Occasional</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20.40</td>
<td>44.35</td>
<td>35.25</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Fungal counts in medical wards of the first period of study ranged between average 26 and 79 cfu / m$^3$, and average counts during second and third period of study was 41 and 122 cfu / m$^3$ and 18 and 119 cfu / m$^3$ respectively. Data showed that the fungal counts in the medical wards during the second period of study is very high than the other set of studies. The second set of study was carried out during June to August when rainfall and relative humidity is higher. When the fungal counts in different wards were compared between the three set or period of studies, data indicated that wards like Casualty (CAS), Female Medical Ward (FMW), Male Medical Ward (MMW) and Pediatric Ward (PED) exhibited higher counts during autumn than summer. (Table-1). In general there is a significant difference in total fungal counts between wards that Casualty, FMW, MMW, PED, GOT and ICU.(P <0.05).

Fungal diversity in the first set of study ranged 2 and 12 different colonies, in second set of study it was ranged between 0 and 13. However, fungal diversity in the third set of study ranged between 0 and 7 different colonies. Data revealed that fungal diversity in the wards during summer and autumn were higher than winter. (Table 2).
A preliminary assessment of Aerofungal Allergens from the wards of civil hospital Aizawl

Table 2: Range number of colonies and diversity of isolated fungi / m³ from six medical wards of Aizawl Civil Hospital

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Wards</th>
<th>No. of beds</th>
<th>Range no. of colonies/ Diversity of colonies.</th>
<th>SET-I / Summer</th>
<th>SET-II / Autumn</th>
<th>SET-III / Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CAS</td>
<td>04</td>
<td>21-140 / 3-10</td>
<td>40-323 / 4-12</td>
<td>30-230 / 3-5</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>PAED</td>
<td>39</td>
<td>0-131 / 4-12</td>
<td>25-327 / 0-5</td>
<td>36-194 / 4-7</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>MMW</td>
<td>34</td>
<td>40-107 / 1-6</td>
<td>19-212 / 3-10</td>
<td>23-238 / 3-6</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>FMW</td>
<td>21</td>
<td>31-145 / 3-9</td>
<td>38-255 / 4-13</td>
<td>24-256 / 4-6</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>ICU</td>
<td>05</td>
<td>0-38 / 2.5</td>
<td>0-75 / 0-5</td>
<td>0-61 / 0-3</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>GOT</td>
<td>04</td>
<td>5-60 / 2.5</td>
<td>26-158 / 2-5</td>
<td>24-94 / 2-5</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Total no. of air samples:</td>
<td></td>
<td>12</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Total no. of colonies (% Contribution)</td>
<td></td>
<td>621**(20.40%)</td>
<td>1350*(44.35%)</td>
<td>1073*(35.25%)</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Mean CFU / m³ (± 1 SE)</td>
<td></td>
<td>103.50 ±18.26**</td>
<td>225 ± 40.03*</td>
<td>178.83 ± 33.36*</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>% of C.albicans from total isolates.</td>
<td></td>
<td>1.05**</td>
<td>3.87*</td>
<td>3.61*</td>
<td></td>
</tr>
</tbody>
</table>

*Denote no significant differences between seasons.

** Denote significant difference between season when compared to autumn and winter. (P<0.05)

During all the three set of studies, filamentous fungi were distributed at considerable levels in all medical wards with variation among different species of isolated fungi. *Aspergillus* species 10.34% of the total isolated fungi, the next proportion are of *Penicillium* species (10.11%) was isolated and other fungi are followed by *Curvularia, Geotrichum, Fusarium, Alternaria* species etc.

Distribution of airborne yeasts were different during all the set of studies with *Candida* species (8.54%) being recognized. During autumn and winter *Candida* species were isolated in proportion of 3.87% and 3.61 % respectively which are higher than the proportion of distribution in summer that was 1.05%. (P <0.05).

Hospital environments are complex environments because they contain different types of microorganisms. Airborne microorganisms are one of these microbes and their presence, numbers and types can indicate the degree of cleanliness of these environments. There are wide varieties of factors which influence airborne counts, and therefore influence hospital infection rates (Jaffal et al 1997; WHO,2002).The evaluation of count, types and diversity of biocontamination in hospital rooms especially OT, ICU are very important to control and prevent hospital acquired infections (HAI).

Results showed that fungal counts in all studied medical wards during autumn are higher than during summer and winter. The higher number of fungi in wards in these seasons may be related to occupant density, temperature and level of humidity. In addition, the bioaerosols containing microorganisms may reside in autumn for long time in air than summer.
The fungal counts in GOT and ICU showed that the quantity of fungi / m³ area was considerably low. This may be due to high sanitary standards in GOT and ICU. However, the high levels of contaminations in other wards such as CAS, FMW, MMW, PED are may be due to outer contamination, more number of beds and the attendant of the patient and their activities.

Diversity of fungi is usually related to the count (Spengler et al., 1983). Data showed that more fungal diversity was found in autumn than in summer and winter.

Studying airborne fungal spores is important to understand dissemination, spread and movement of the microbes, particularly the pathogenic ones in the atmosphere (Saad., 2003). During autumn and winter the percentage of yeasts increased while the mold decreased in summer. These results may be correlated with high level of humidity in autumn and winter than in summer. (Begs.2003)

The common genera of fungi that frequently isolated from the hospital wards are Aspergillus species, Penicillium species, Curvularia, Geotrichum, Fusarium, Alternaria, Drechslera, Cladosporium, Scolocobasidium, Torula and Scopulariopsis. However, the common genera of yeast that are frequently isolated from medical wards are Candida species. Also a significant number of Aspergillus species (10.34%), Penicillium species (10.11%), Curvularia species (9.19%) and Candida species (8.54%) were found in comparison with other species.

Aspergillus and Penicillium species were common in their occurrence. Alternaria, Curvularia, Fusarium, Geotrichum and yeast Candida species were exists frequently in samples. Fungi like Cladosporium, Drechslera, and Scolocobasidium species were isolated from wards occasionally. Then the rest of the fungal species are recorded only sporadically from the atmosphere of Aizawl Civil Hospital wards (Figure 2).

![Figure 2: Percentage distribution of Fungi flora recorded from the wards of Civil Hospital Aizawl during 2010 in three different climatic conditions.](image-url)

A total of 13 fungi genera were recorded from the atmosphere of Civil Hospital Aizawl. Among them a majority belongs to Deuteromycotina. The predominance of Deuteromycotina
spores from the atmosphere of different cities was reported worldwide. The details of genera identified from Civil Hospital Aizawl, Aizawl, Mizoram is given in table number – 1.

Although a large number of fungi were recorded, a great proportion of them were sporadic in their occurrence. Thus, depending on the percent isolation frequency the moulds were grouped as ‘common’, ‘frequent’, ‘occasional’ and ‘sporadic’. Such grouping was earlier made by Satheesh Kumar (1999); Bhuvaneswari (2005) and Banu Rekha (2006).

Aspergillus species contributed highest to the total aerospora in all the medical wards in all the seasons (Table-1) corroborating to Jaffal (1997), Verma et al (2003), Manuel et al. (1998). Present study showed the similar distribution of the mold and yeast species like Aspergillus, Penicillium, Curvularia, Geotrichum, Fusarium species have the major share to the total aerospora.

The present study clearly indicates that the high relative humidity, low degree of temperature during autumn and winter have a strong influence on high distribution and count of fungal isolates which were isolated from the atmosphere of Aizawl Civil Hospital, Mizoram.

5. Conclusion and recommendations

5.1 Conclusion

In conclusion, airborne concentrations of fungi in six different medical wards of Aizawl Civil Hospital in north eastern India indicated higher degree of contamination but less diversity during autumn than during summer. The high sanitary standards in OT and ICU are major reasons of the low level of contamination.

5.2 Recommendations

In the context of healthcare settings, bio-aerosols can cause occupational hazards and nosocomial infections. Bio-aerosol monitoring in hospitals can be used for tracking of nosocomial infections, identify the source and spread of airborne microorganisms to control Hospital Associated Infections (HAI). This will also serve as a tool to measure biosafety while handling biohazardous materials. In order to reduce bio-aerosol loads in indoor environments, certain control measures can be followed. These include proper identification and elimination of the microbial sources in occupational and house-hold settings, maintenance of equipment, humidity control, natural ventilation, use of filters in ventilation, and air cleaning by the use of disinfectants and biocides. Periodical use of disinfectants and biocides is one of the most common methods to ensure controlled bio-aerosol concentrations. Air in the operating rooms and others critical areas like isolation rooms can be disinfected by fumigation using various microbicidal agents. (www.pollutionissues.com).

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6. References


