AIM OF THE STUDY

To evaluate the prevalence of airborne Aspergillus spores within a hospital environment and its correlation with clinical isolates, if any.

INTRODUCTION

Invasive aspergillosis (IA) is an airborne disease, patients usually acquire infections by inhalation of spores. Depending on the clinical status, person either colonizes the spores initially, then gets infections. In recent years there has been an inexorable increase in number of immunocompromised patients in the hospital environment. There is also another group of patients without severe immunosuppression still they are at risk of invasive fungal infections. High concentration of aspergillus spores in the hospital or renovation work inside or adjacent to the hospital environment is major risk factor for acquiring the infections.

METHODOLOGY

A total of one hundred and ninety-five air samples from high-risk areas hematology, pulmonary wards & intensive care units were collected during the study period from May 2017 to May 2018, during the study period a total of 282 clinical samples (BAL, Sputum, Tissue, ETA, Pus) were collected from patient suspected of invasive aspergillosis based on clinical sign symptoms and radiological imaging findings.

The air samples were collected with centrifugal sampler at a air flow rate of 100L/min (total 500L) onto potato dextrose agar plates, incubated at 37°C for 48hr with a mean count of 25.6 cfu/m³.

Clinical samples from patients with suspicion of invasive aspergillosis were processed by conventional mycological methods.

According to EORTC/MSG criteria most of the patients were classified as probable cases of aspergillosis.

259 isolates from 190 air sample and 181 clinical isolates were included in the final analysis since culture was positive in same time.

Genomic DNA was extracted by chemical & mechanical lyses of fungal wall by liquid nitrogen method followed by silica column purification (Ref.1).

RAPD PCR was performed using three primers: R108 (GATTTGCCCT), R151 (GCTGTAGCTC) and UBC90 (GGGGTTAGG). PCR product was separated on 1.5% agarose gel stained with ethidium bromide (Ref.2).

Dendogram was constructed using UPGMA algorithm with PyElph ver 1.4

RESULTS

Based on morphological features, 76 of the 181 isolates from patients were identified as A. fumigatus (42%), 86 as A. flavus (48%), and 19 as A. niger (10%) as shown in Table 1. Moreover, some of the Aspergillus isolates from the clinical and hospital indoor were completely matched (matched pairs) but only few isolates showed similar RAPD pattern by each individual primer. (Fig 1, 2 and 3). In total, 8 A. fumigatus (4 clinical, 4 environmental), 4 A. flavus (2 clinical, 2 environmental), and 2 A. niger (1 clinical, 1 environmental) matched isolates were found suggesting that every isolate present in the environment is potential pathogen if encountered with appropriate host. The major RAPD type for all the primers is Type I (Table 2). Of the three primers used, primer UBC90 displayed the highest degree of discriminating power (D=0.740), detecting three RAPD types. Primers R108 and R151 both detected two RAPD types with D=0.527 and D= 0.264 respectively.

DISCUSSION

There was unremitting construction going on outside the ward and ICU. In order to find out the nosocomial origin of IA this study was conducted. RAPD PCR has been examined as a typing method for monitoring the source of infection (community acquired vs. nosocomial) due to its technical simplicity as no previous sequence information is needed, in contract with other techniques like RFLP typing. The three primers used in our study has been earlier used successfully for A. fumigatus typing (Ref. 3). All the matched clinical isolates of A. fumigatus and A. flavus were taken post air sampling from same ward/ICU within same month except for A. niger, the clinical sample was received before air sampling. Nevertheless, the RAPD pattern for A. niger matched almost with respective air sample isolate suggesting the possible role of nosocomial infection in the patient. We excluded patient-to-patient transmission because of the different time intervals of hospitalization of the patients. Moreover, use of mixed primer pairs will further confirm the occurrence of IA within a hospital environment and its correlation with clinical isolates.

CONCLUSION

Though genetic analysis cannot discriminate absolutely between clinical and environmental isolates. Both the identity of fungal species in clinical and environmental samples indicates that every isolate present in the environment is potential pathogen if encountered with appropriate host. Routine environmental surveillance of high-risk areas for fungi may help to control the limit the contamination. It will further help to reducing the incidence of fungal infections.

REFERENCES


ACKNOWLEDGEMENT: We thank the clinicians and other health care workers for their unique cooperation and also the mycological laboratory staff for their support and help during this study.

Table 1: No. of isolates obtained from clinical and air samples

<table>
<thead>
<tr>
<th>Species</th>
<th>Clinical samples</th>
<th>Air samples</th>
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<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>76</td>
<td>139</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>86</td>
<td>87</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>19</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>181</td>
<td>259</td>
</tr>
</tbody>
</table>

Table 2: Number of types from RAPD analysis with clinical and air samples

<table>
<thead>
<tr>
<th>Primer</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>R108</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R151</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UBC90</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

Fig 1a, RAPD typing of clinical and environmental isolates by using primer R108. Marker. 100bp (M), Lanes C1-C4, A. fumigatus clinical isolates, E8, E11, A. flavus, environmental isolates, Lane D5-D6, A. flavus, clinical isolates. 48hr. 100bp \( \text{GTATTGCCCT) and UBC90 (GGGGTTAGG).} \) PCR product was separated on 1.5% agarose gel stained with ethidium bromide.

Fig 1b, Dendogram of RAPD analysis showing various RAPD types.

Fig 2a, RAPD typing of clinical and environmental isolates by using primer R151. Marker. 100bp (M), Lanes C1-C4, A. fumigatus clinical isolates, E8, E11, A. flavus, environmental isolates, Lane D5-D6, A. flavus, clinical isolates. 48hr. 100bp \( \text{GGGGTTAGG) and UBC90 (GGGGTGTTAGG).} \) PCR product was separated on 1.5% agarose gel stained with ethidium bromide.

Fig 2b, Dendogram of RAPD analysis showing various RAPD types.

Fig 2c, RAPD typing of clinical and environmental isolates by using primer UBC90. Marker. 100bp (M), Lanes C1-C4, A. fumigatus clinical isolates, E8, E11, A. flavus, environmental isolates, Lane D5-D6, A. flavus, clinical isolates. 48hr. 100bp \( \text{GGGGTTAGG) and UBC90 (GGGGTGTTAGG).} \) PCR product was separated on 1.5% agarose gel stained with ethidium bromide.

Fig 3a, Dendogram of RAPD analysis showing various RAPD types.

Fig 3b, Dendogram of RAPD analysis showing various RAPD types.

Fig 3c, Dendogram of RAPD analysis showing various RAPD types.