# RELATIONSHIP BETWEEN CLINICAL AND ENVIRONMENTAL ASPERGILLUS ISOLATES FROM THE HIGH-RISK AREAS OF A TERTIARY CARE HOSPITAL

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## AIM OF THE STUDY

To evaluate the presence 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 highly 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 $37^0$C for 48hr with a mean count of 26.5 cfu/$m^3$
- 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 (GTATTGCCCT), R151 (GCTGTAGTCT) and UBC90 (GGGGGTTAGG). 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=0.704), detecting three RAPD types. Primers R108 and R151 both detected two RAPD types with D=0.527 and D= 0.264 respectively.

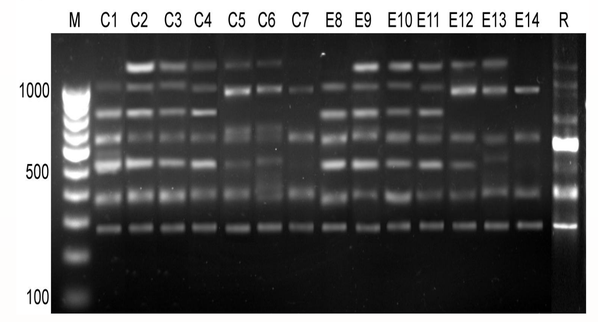
**Table 1: No. of isolates obtained from clinical and air samples**

| Species | Clinical samples | Air samples |
|---|---|---|
| *Aspergillus fumigatus* | 76 | 139 |
| *Aspergillus flavus* | 86 | 87 |
| *Aspergillus niger* | 19 | 33 |
| Total | 181 | 259 |

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

| Primer | RAPD Types, *Aspergillus fumigatus (n=8)* | | |
|---|---|---|---|
| | Type I | Type II | Type III |
| R108 | 2 | 6 | 0 |
| R151 | 8 | 0 | 0 |
| UBC90 | 2 | 2 | 4 |
| | **RAPD Types, *Aspergillus flavus (n=4)*** | | |
| R108 | 2 | 2 | 0 |
| R151 | 2 | 2 | 0 |
| UBC90 | 2 | 2 | 0 |
| | **RAPD Types, *Aspergillus niger (n=2)*** | | |
| R108 | 2 | 0 | 0 |
| R151 | 2 | 0 | 0 |
| UBC90 | 2 | 0 | 0 |

**1(a)**




**1(b)**

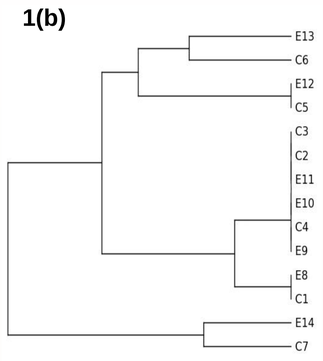
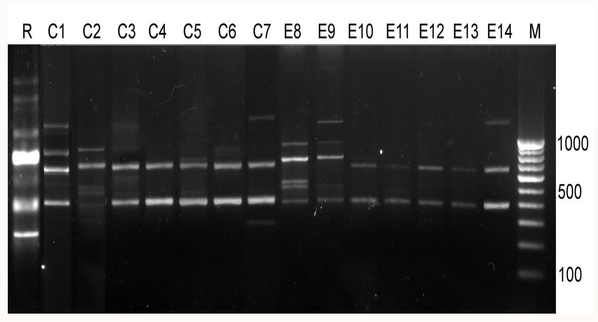



Fig 1(a). RAPD typing of clinical and environmental isolates by using primer R108. Marker 100bp (M). Lanes C1-C4, *A. fumigatus*, clinical isolates. E8-E11, *A. fumigatus*, environmental isolates. Lanes C5-C6, *A. flavus*, clinical isolates. E12-E13, *A. flavus*, environmental isolates and Lane C7, *A. niger*, clinical isolates. E14, *A. niger*, environmental isolates. Lane R, *A. fumigatus* reference strain. (b). Dendogram of RAPD analysis showing various RAPD types.

**2(a)**




**2(b)**

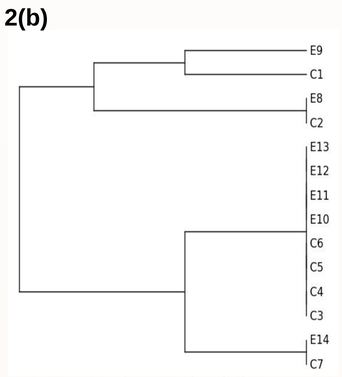
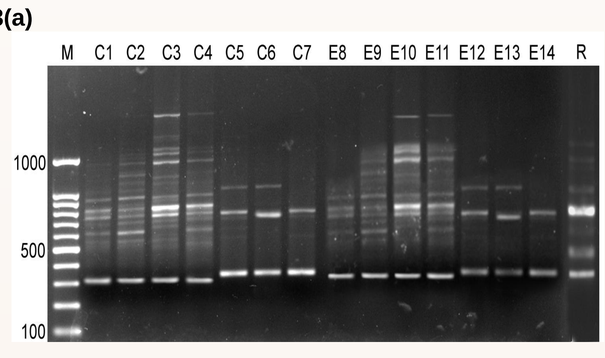



Fig 2(a). RAPD typing of clinical and environmental isolates by using primer R151. Marker 100bp (M). Lanes C1-C2, *A. flavus*, clinical isolates. E8-E9, *A. flavus*, environmental isolates. Lanes C3-C6, *A. fumigates*, clinical isolates. E10-E13, *A. fumigatus*, environmental isolates and Lane C7, *A. niger*, clinical isolates. E14, *A. niger*, environmental isolates. Lane R, *A. fumigatus* reference strain. (b). Dendogram of RAPD analysis showing various RAPD types.

**3(a)**




**3(b)**

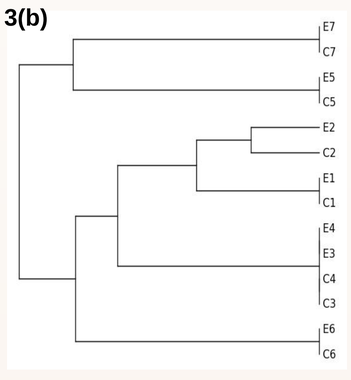



Fig 3(a). RAPD typing of clinical and environmental isolates by using primer UBC90. Marker 100bp (M). Lanes C1-C4, *A. fumigatus*, clinical isolates. E8-E11, *A. fumigatus*, environmental isolates. Lanes C5-C6, *A. flavus*, clinical isolates. E12-E13, *A. flavus*, environmental isolates and Lane C7, *A. niger*, clinical isolates. E14, *A. niger*, environmental isolates. Lane R, *A. fumigatus* reference strain. (b). Dendogram of RAPD analysis showing various RAPD types.

## 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

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3. Lin D, Lehmann P, Fhamory BH: Comparison of three typing methods for clinical and environmental Isolates of *Aspergillus fumigatus*. J Clin Microbiol 1995; 33 (6): 1596-1601.

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