RESEARCH ARTICLE

INVESTIGATION ON AIRBORNE MOULDS IN GOVERNMENT HOSPITAL WARDS OF WARUD CITY, AMRAVATI DISTRICT

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ABSTRACT

The airborne culturable moulds and spor types in the indoor environment of Govt. hospital wards of Warud were monitored over one year period from August 2011 – November 2012, using a two stage Andersen air sampler and Burkard personal slide sampler. Among culturable moulds *Cladosporium herbarum* and *Penicillium* citrinum were most frequently recorded. Aspergilli/Penicilli among airborne conidial types was most frequently recorded. The percentage of occurrence of *mycoflora* was higher in General Ward in comparison to Post Surgical Ward of hospital.

Keywords : Airborne moulds, *Cladosporium herbarum, Penicillium citrinum, mycoflora.* Warud.

INTRODUCTION

Environment plays an important role in the precipitation of allergic symptoms. With rapid industrialization and urbanization the air is becoming polluted with different bio aerosols and this has increased the risk of allergic disorders. Many surveys have been conducted to elucidate the magnitude of the problem. Since the 1960's the prevalence of asthma has been increasing and this increased prevalence is associated with a high degree of sensitization to inhalant allergens. (*Pandey & Mishra,* 2011) Many airborne fungal spores are important as allergens, human and plant pathogens and in the bio-deterioration of stored materials as well as spoilage of food stuffs.

The vast and diverse geographical region essentially demands the preparation of a list and calendar of allergenic fungal spores, since the allergens differ to a certain extent from region to region. India being located between 7 0 N to 63.6 0N and 67 0 E to 98 0 E, is bestowed with a wide range of biomass. Such diversity in the vegetation contributes an enormous variation in the quality and quantity of airborne fungal spores from different parts of India. Accordingly, the incidence and symptomatology of allergic patients from different parts of India is extremely variable. Despite this, no comprehensive survey of mould spores in hospital wards of Warud city is available. Hospitalized patients have a potential risk for nosocomial infections (*Martins-Diniz* et al., 2005). Airborne fungi are one of the main causes of fungal infections in this group. The objective of this study was to investigate the concentration and species of airborne fungi in hospital wards of Warud city of Amravati District. Hence, for the first time a qualitative and quantitative survey of atmospheric fungal spores of hospital wards of Warud city of Amravati District was undertaken and the results of which are described in this communication.

MATERIAL AND METHODS

Air sampling of hospital wards was carried out by using together an Andersen sampler and a Burkard Personal Volumetric sampler. Three samples approximately at ten days interval in a month were

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taken from General ward and Post-surgical wards of hospital from August 2012 to July 2013.

1. Andersen Sampler: Two stage Andersen's sampler was used. Petridishes containing Streptomycin Rose Bengal Agar medium (Martin, 1950) were used for exposure in the sampler and the duration of sampling was 2 minutes. Sampling was done at 2 feet above the ground.

After exposure the Petridishes were brought back to the laboratory in a pre- sterilized polythene bags and incubated at 25±20C temperature. A total of 18petriplates were exposed every month in each ward from August 2012 to July 2013. The different species of fungi appearing on isolation plates were sub cultured and identified.

The fungal isolates were characterised and identified according to the manual of Barnett and Hunter (Barnett and Hunter, 1972). A complete record of data regarding the total number of colonies per plate and the total number of colonies of each species in a petriplate was recorded. After entry of monthly data the average fungal colony counts have been converted in to number values using the conversion factor (15.87). Per cent concentration of each fungal spore was calculated.

2. Burkard Volumetric Personal Air Sampler: Sampling was also carried out with a Burkard Volumetric Personal Air sampler. The sampler with a suction rate of 10 L/min. was operated for 5 minutes.

The air borne particles (*mycoflora*) were deposited on micro slides smeared with cotton blue stained glycerine jelly. The sets of exposed slides were brought back to the laboratory in a sterilized covered Petridishes. Each slide was warmed gently over flame to remove the moisture. The dust particles, soot particles and insect parts were removed by sterilized dissecting needles and forceps using a hand lens. The slide was then mounted with a cover glass. Only fungal spores under the cover glass were taken in to account for the counts. Spore types were identified up to the generic level with microscopic methods, according to Barron (1972). As spores of Aspergillus and *Penicillium* could not be distinguished on the basis of morphology therefore they were grouped collectively under the category 'Asp/Pen'. The counts are expressed as number per cubic meter of air by using conversion factor (71.43).

3. Statistical analysis: The data were analysed by Pearson's test for correlation.

RESULTS

1. Andersen Sampler:

During studies on indoor air of hospital wards a total of 26 species belonging to 10 genera were isolated from both the wards i.e. General ward and Post-surgical ward. The percentage of occurrence of *mycoflora* was higher in General ward (51.93 %) in comparison to Post surgical ward (48.06 %). The highest number of Colony Forming Unit (CFU) was recorded for *Cladosporium herbarum* (3681.84 CFU/m 3) followed by *Penicillium* citrinum (3062.91CFU/m3), Aspergillus niger (2126.58 CFU/m3) and Aspergillus flavus (1047.42 CFU/m 3) (Table-1). Highest per cent concentration of the spores was recorded in the month of January (17.21%) followed by February (16.84%), March (14.75%) and December (13.81%). While least per cent concentration of spores was recorded in the month of July (1.41%). All the 26 species were isolated from both the wards. The spores of Alternaria alternata, Aspergillus niger, *Cladosporium herbarum*, C. Oxysporum and *Penicillium* citrinum were recorded throughout theyear. *Cladosporium herbarum* was numerically the most abundant in both the wards. Its contribution was highest in the Post-surgical ward and least in General ward.

2. Burkard Volumetric Personal Air Sampler:

The total aero*mycoflora* encountered in the atmosphere over the year was 68916 fungal spores/m3 Distributed amongst 10 types. 'Asp /Pen' (52.43%) constituted the predominant spore type. Cladosporium (27.97%) was other numerically significant type recorded in annual calendar. The highest number of fungal spores were present in December (8641.82 spores/m3) and lowest in April (3356.74 spores/m3). The spores of 'Asp/Pen' and Cladosporium were recorded throughout the year.

Most frequently trapped spores were Alternaria and Curvularia sp. Highest number of spores were recorded from General ward in comparison to Post surgical wards. Highest no. of spores was recorded.

Table 1. Fungal colonies calendar showing the annual total for fungal colonies from August 2012 to July 2013.

Fungal spores	No. of CFU / m3												Total
	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Mar	Jun	Jul	
Absidia sp.	-	-	-	-	-	47.61	63.48	79.35	-	-	-	-	190.44
Alternaria alternate	63.48	-	-	47.61	-	-	31.74	-	15.87	63.48	47.61	47.61	317.4
A. carthami	-	-	-	-	-	47.61	-	-	-	-	-	-	47.61
A. dianthi	-	-	-	-	-	79.35	95.22	79.35	-	-	-	-	253.92
A.dianthicola	-	-	-	-	-	-	111.09	79.35	-	-	-	-	190.44
A. geophila	-	-	-	-		63.48	63.48	-	-	-	-	-	126.96
A. humicola	-	-	-	-		95.22	79.35	63.48	-	-	-	-	238.05
A. radicina	-	-	-	-	111.09	95.22	63.48	-	-	-	-	-	285.66
A. sonchi	-	-	-	-	126.96	111.09	-	79.35	-	-	-	-	317.4
A. tenuissima					126.96	95.22	-	79.35	-				301.53
Alternaria sp.						-	79.35	-	95.22	111.09			285.66
Aspergillus candidus				190.44	126.96	-	-	-	-				317.4
A. flavus				158.7	174.57	190.44	158.7	238.05	126.96				1047.42
A. koningi					31.74	-	47.61	-	63.48				142.83
A. niger	79.35	63.48	174.57	158.7	190.44	285.66	317.4	396.75	238.05	95.22	126.96	-	2126.58
A. sydowi			111.09	-	396.75	317.4	238.05	285.66	190.44				1539.39
Chaetomium cristatum						63.48	31.74	-	-				95.22
C. globosum						63.48	47.61	31.74					142.83
Cladosporium herbarum	-	634.8	507.84	396.75	428.49	396.75	317.4	285.66	238.05		476.1	-	3681.84
C. oxysporum		126.96	158.7	190.44	111.09	126.96	79.35	95.22	-			79.35	968.07
Curvularia lunata						126.96	79.35	47.61	31.74				285.66
C. pallescence					63.48	79.35	63.48	47.61-	-				253.92
Drechslera australiensis					47.61	31.74							79.35
Fusarium oxysporum						47.61							47.61
Penicillium citrinum	111.09	126.9	158.7	-	317.4	396.75	634.8	714.15	396.75	95.22	-	111.09	3062.91
Syncephalastrum					126.96	111.09	95.22	-	95.22				428.4

Spore types identified from Burkard sampler from August 2012 to July 2013. in the month of December followed by October, November and January. Least number of spores was recorded in the month of April.

3. Statistical analysis:

Results of statistical analysis suggest that the *mycoflora* of general ward might be very positively associated with *mycoflora* of post surgical ward. The results showed that, the fungi load were significantly not different for the wards studied.

DISCUSSION AND CONCLUSION

A combination of two techniques, viz. Andersen sampler and Burkard volumetric air sampler has been used in the present investigation to get a fairly complete picture of the air *mycoflora* of the hospital wards. In fact, such a combination for sampling indoors was suggested by earlier worker 5 who emphasized the need of using two trapping methods during aerobiological surveys, one for the microscopic assessment of the total air spora and the other for the identification of predominant types in culture. Twenty six species of fungi, belonging to Deuteromycetes, were isolated and identified from the General and Post- surgical wards of hospital by Andersen sampler which included many potential allergens. Species of *Aspergillus, Alternaria, Cladosporium* and *Penicillium* were most frequently isolated from indoor air of hospital wards.

The presence of aspergilli in the hospital environment is a major extrinsic risk factor for the occurrence of nosocomial invasive aspergillosis (*Martins-Diniz* et al., 2005; Martin, 1950; Barnett and Hunter, 1972; Barron, 1972; Tilak, 1982; Dutkiewicz and Augustowska, 2006; Sessa et al., 1996). The number and types of fungus spores in indoor air seem to depend on air exchange with the outside and the presence of indoor spore sources like patients, visitors etc (Lacey, 1981; Caretta, 1992; Wu et al., 2005; Sandra et al., 2009). *Cladosporium herbarum* was recovered in large number from the Post-surgical

ward. Cladosporium grow well indoors in fiberglass insulation or highly humid surfaces (Ghani, 1997). Many reports indicated that this genus is the predominant fungus and one of the most common isolates cultured from inside the hospitals which are in keeping with our results. The predominance of Aspergillus and *Penicillium* in the air of indoor environments of hospital wards was established by earlier workers (Ghani, 1997; Ekhaise et al., 2008; Hedayati and Mohamad, 1999; Pakshir et al., 2007).

Predominance of Cladosporium, Aspergillus and *Penicillium* has also been confirmed by Burkard sampler data. High frequency of Cladosporium in General ward of hospital was revealed by Burkard sampler. Spores of Beltrania and Ulocladium found on Burkard trap slides could not be recovered on the plates of Andersen sampler. In both the methods i.e. cultural (Andersen sampler) and noncultural (Burkard sampler) the isolated genera were same because of this may be the indoor air depends upon the outside ventilation. The highest per cent concentrations of spores were recorded from General ward in comparison to Post surgical wards. The reason of this may be, because of restricted entry of visitors, more disinfected area of the hospital and closed and controlled condition of the ward.

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