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AN AEROMYCOLOGICAL STUDY FOR ASPERGILLUS IN THE RESPIRATORY MEDICINE AND CHEST DISEASE WARDS OF A TERTIARY CARE HOSPITAL, AJMER(RAJ.)

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ABSTRACT **Introduction:** The indoor air quality of healthcare institute is highly concerned, as high percentage of air borne fungal spores act as a potential risk factor to the people reside inside. Fungal infections comprised 9% of nosocomial infections whereas *Aspergillus* is the second most common cause with 1.3%. **Material and methods**: A total of 432 air samples (RMCD wards n =168, control sites n= 264) and 24 AC filter dust samples were collected every fortnight from Jan 2018 to Dec 2018 to monitor the frequency of *Aspergillus* spores. Air samples were collected by using sieve type air sampler. **Results:** The mean spore count of *Aspergillus* in RMCD wards and control sites were 22.25 and 12.76 respectively. Mean spore count was more in non air-conditioned(26.58) compared to air-conditioned wards(9.25). Out of 56 cases, 57% were suspected to be suffering from nosocomial infection. **Conclusion:** The presence of high percentage of *Aspergillus* spore load in air of indoor wards act as a potential risk factor for allergic, asthma, ABPA, invasive disorders and nosocomial infections which highlights the need to ensure use of standard regular housekeeping activities.

KEYWORDS : Aspergillosis, Nosocomial infections, Czapek Dox Agar, *Aspergillus fumigatus*

INTRODUCTION-

Respiratory tract infections are globally responsible for one-third of infectious disease–associated mortality, accounting for 4.3 million annual deaths[1].Patients suffering from tuberculosis (TB), Chronic obstructive pulmonary disease (COPD), asthma etc. are more prone to fungal infections especially aspergillosis because of long term treatment and steroids therapy [2-5]. Fungal infections comprised 9% of nosocomial infections whereas *Aspergillus* is the second most common cause with 1.3%[6-8].The indoor air quality is highly concerned, as high percentage of air borne fungal spores act as a potential risk factor to the people reside inside that may cause various respiratory diseases from allergy, superficial, invasive infections to nosocomial infections[9].The majority of the fungus found in the interior environment of health care institutions are nonpathogenic or opportunistic pathogens. Due to increasing number of immunosuppressed and severely ill patients in the hospital setting it become necessary to know the burden of *Aspergillus* in the air. The primary route of infection in cases of aspergillosis by inhalation of fungal spores[7,10].Pneumonia is the most common clinical presentation for aspergillosis with very high mortality rate as high as 95%.The most common causative agents of nosocomial *Aspergillus* infection are *Aspergillus fumigatus*, *Aspergillus flavus* followed by other species[7,11].The fungal species make up the majority of airborne flora. The number of spores in indoor air varies depending on meteorological factors, climate, cleaning, ventilation systems, age of the buildings, no. and frequency of movement of people[12-13].Hence this study was designed to determine the concentration of *Aspergillus* spores to monitor the air quality in the Respiratory Medicine and Chest Disease (RMCD) wards, so as to ensure well-being of patients and healthcare workers and also to know airborne source of nosocomial infections due to different *Aspergillus* species.

MATERIALS AND METHODS:-

This was a prospective, observational, ecological study. The study was conducted in 60 bedded RMCD hospital. A total of 432 air samples(RMCD wards, n=168) and control sites, n= 264) and 24 AC filter dust samples were collected every fortnight over a period of one year from Jan 2018 to Dec 2018 to monitor the concentration of *Aspergillus* spores. During this period a total of 6574 patients were admitted in the RMCD wards of J.L.N.Hospital Ajmer. A total of 184 clinically suspected samples of fungal infection were received in the department of microbiology. Out of which 56 patients were those who develop clinical signs and symptoms of aspergillosis after 48hr of admission. Therefore only these patients were included in this study as they were suspected to be suffering from nosocomial infection. A total of 108 samples were received from 56 patients (sputum n=52 in duplicate and BAL n=4). Air samples were collected by using sieve type air sampler (Hi media model no.LA881) by collecting 100L/ min for 10 min on 90 mm petri dish of Malt Extract Agar(MEA),Czapek Dox Agar (CDA) and Sabouraud Dextrose Agar(SDA) with chloramphenicol. After sample collection, plates were incubated at 22°C till the time the colonies were countable. To evaluate the air biocontamination level due to *Aspergillus* sp. of the RMCD wards and control environment area, colony counts per cubic meter of air was calculated using formula[14]:

$$\text{CFU/m3} = \frac{\text{No. of fungal colonies on SDA plate at the end of 3 days/later(if countable)}}{\text{Total volume of air sampled in m3}}$$

Fungal growth on the plates were noted, subcultured on fresh SDA plates and incubated at both 22ºC and 37ºC to rule out dimorphic fungi. Clinical samples (sputum and BAL) were inoculated on SDA with chloremphenicol and incubated at both 22ºC and 37ºC. Identification of all the isolates were done using phenotypic methods by standard mycological procedures. Macroscopic identification was done on the basis of growth rate, colony morphology, color and texture while microscopic identification was done on the basis of shape and size of conidia, vesicle and conidiophore in LPCB mount. All culture media were obtained from HiMedia laboratories Pvt.Ltd, Mumbai, India.

Statistical analysis

Quantitative data were expressed as mean using SPSS, Version 21.0(IBM Corp, Armonk, NY, United States).

RESULTS:-

The annual spore index of *Aspergillus* was 723 in RMCD wards. The monthly spore index was maximum in Feburary (n= 162) and minimum in August and September (n = 0 & n= 8) (Table 1).The total CFU/m^3 ranged from 1 to 234 CFU/m^3 in RMCD wards and 1 to 136 CFU/ m^3 in community. The fungal counts in indoor and outdoor environment were highest in winter season. Fungal load was less in AC rooms compared to Non AC rooms. The mean spore count of *Aspergillus* was 22.25 in RMCD wards and 12.76 in control samples across different seasons while 26.58 in non air-conditioned and 9.25 in air-conditioned wards. The predominant species was *Aspergillus niger* which occurred at high frequency throughout the year, with little evidence of seasonal variation (Fig1). The maximum no of *Aspergillus* spores were isolated from MMDR ward while minimum no. were reported from the female ward (Table-2).

Czapek Dox Agar was found to be best media for the air sampling of *Aspergillus* sp. isolation. Out of 56 clinical samples only 32(57%) samples were confirmed culture positive for *Aspergillus* and all were isolated from sputum samples. The following *Aspergillus* spp. were

isolated from clinical samples *As.niger*(44%), *As.fumigatus*(41%) and *As.flavus*(16%) while from indoor environment of RMCD wards 723 *Aspergillus* sp. were isolated *As.niger* (69%), *As.flavus* (16%), *As.fumigatus*(0.13%), *As. nidulans*(2%), *As.glaucus*(0.69%), *As.ustus*(0.27%) and *As.sp* (12.44%).The clinical *Aspergillus* isolates which were phenotypically resembling with the environmental isolates of RMCD wards were considered to be suspected cause of nosocomial infection.

Table 1: Monthly spore index of *Aspergillus* isolates in RMCD wards, Outdoor and Indoor control environment

Months	As.load (cfu/m3) in RMCD Wards	As.load (cfu/m3) in Outdoor Control	As.load (cfu/m3) in Indoor Control
January	70	4	89
February	162	56	43
March	83	31	10
April	33	30	23
May	72	17	9
June	67	30	0
July	58	26	10
August	0	12	0
September	8	12	0
October	42	123	110
November	34	33	34
December	94	129	126
TOTAL	723	503	454

Fig 1: Seasonal distribution of CFU of *Aspergillus* sp.in different sites

Table 2: Site wise fungal load of *Aspergillus* isolates in RMCD wards, Outdoor and Indoor control environment

RMCD Wards	As. load in cfu/ m3	OUTDOOR Control	As. load in cfu/m3	INDOOR Control	As. load in cfu/m3
MMDR	188	Anasagar	105	Home 1	149
FMDR	124	Shastri nagar	149	Home 2	105
Male A	97	Madar gate	5	TBCD OPD	193
Male B	78	Dhola bhata	55	MICRO LAB	7
Male C	95	Subash nagar	35		
ICU	75	Outdoor TBCD	153		
Female ward	59	College campus	1		
AC	7				
TOTAL	723		503		454

DISCUSSION:-

The presence of high percentage of *Aspergillus* spp. in the air of the RMCD wards is worrisome. Because the patients of these units are usually severely ill or immunosuppressed, and this group is at the highest risk of suffering from potentially fatal invasive fungal diseases, allergic, asthma, ABPA and mycotoxin related disease[9,15]. They may also prove to be a threat to immunocompetent hosts, such as the healthcare staff.

Aspergillus niger and *Aspergillus flavus* were the most prevalent species in the air than *Aspergillus fumigatus* in both RMCD wards and control environment. The fungal bioload was less in AC ward like ICUs as compared to non AC wards. This may be due to close premises, restricted no. and frequency of movement of people in ICUs. Fungal counts in both indoor and outdoor environment were highest in winter season. This may be due to air being moist because of fog. As moist is favorable for fungal growth, while other authors reported maximum fungal counts during summer and rainy seasons[16-17].The maximum no. of *Aspergillus* spores were isolated from MMDR ward because of open environment and dampness in the wall and ceiling whereas minimum were reported from the female ward. From indoor control environment maximum were reported from TBCD OPD due to more crowd of patients and minimum from microbiology lab while from outdoor control environment maximum no. were reported from shastri nagar due to heavy plantation and minimum from college campus.

Maximum genera of fungi isolated with higher CFU from MEA medium, moderately from SDA medium and minimum from Czapek dox agar. But CDA was found to be best medium for the air sampling of *Aspergillus* as the CFU for *Aspergillus* sp. was maximum on this media and it is also selective media for *Aspergillus* sp.

The no. of fungal spores increased during construction/renovation near hospital hence it increases the chances of airborne fungal infections[10,18].This can be minimize by installing barriers between patient-care areas and construction/ renovation areas using portable HEPA filters and sealing patient windows. Other authors also reported that incorporation of HEPA filter in wards, ICUs and regular cleaning of AC filters helps to reduce the fungal bioload in the indoor environment as such facilities are highly effective in filtering *Aspergillus* conidia and preventing pulmonary aspergillosis thus reduce the chances of nosocomial infections also[18-21].

In the present study the concentration of air borne *Aspergillus* in patient care area ranged from 0-234 spores/m^3 which is worrisome as the spore count <1 CFU/m^3 were sufficient to cause infection in high risk patients[10]. Its high percentage in the air act as a potential risk factor for allergic, asthma, ABPA, invasive disorders and nosocomial infections to patients[9,22]. The incidence of Aspergillosis was maximum due to *As. niger* and *As.fumigatus* though the fungal bioload in the air of wards were more for *As. niger* and *As.flavus* than *As.fumigatus*. More cases of *As.fumigatus* were reported due to its small spore size. The guideline of nosocomial infections also stated that single spore of the *As.fumigatus* can be responsible for causing nosocomial infection. The concentration of specific strain's may fluctuate rapidly, therefore a nosocomial *Aspergillus* infection cannot be excluded even if the strain is not found in the hospital environment[23].Hence to understand the concept of nosocomial infections this type of study should be done for long duration of time and different type of clinical samples should be incorporated in the study. The shortcoming of this study was that only sputum and BAL samples were included and it is unclear whether the patients were colonized or infected before admission hence it is difficult to differentiate either it is colonization or infection.

CONCLUSION: -

The presence of highly pathogenic *Aspergillus* spp. in indoor wards highlights the need to ensure use of standard regular housekeeping activities. High percentage of *Aspergillus* spores in the air act as a potential risk factor for allergic, asthma, ABPA, invasive disorders to nosocomial infections. By using standard regular housekeeping activities, cleaning of AC filters, use of portable HEPA filters controlling dampness in the wall and ceiling helps to decreases the fungal bioload. This study will be useful to spread awareness & initiation of appropriate interventions to make the environment safer for patients and health care workers which will be helpful to decrease the nosocomial infections, morbidity and mortality due to aspergillosis.

Future prospect

Molecular characterization and comparison of fungal isolates from patients and their environment will be done to understand the epidemiology and rule out either it is colonization or nosocomial infection.

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