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Hospital Indoor Airborne Microflora in Private and Government Owned Hospitals in Sagar City, India

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Abstract: The level of airborne microbial load of hospital indoors is unknown in Sagar City, Madhya Pradesh, India. Microbial monitoring of such environments is important for the quality of human life. A study of the quality and quantity of airborne microflora in two major hospitals, the Chaitainya Hospital and District (Tilli) Hospital of Sagar was carried out to establish standard for future reference. Samples were collected using the settled plate technique and '*Hi Air*' air sampler for enumeration of bacterial and fungal isolates. Variety of bacteria, fungi and actinomycetes were isolated using wide range of agar based media like malt extract agar, Sabouraud's dextrose agar, nutrient agar and Czapek's dox agar. Microbial aerobiodiversity as well as microbial load was enumerated in indoor environment of four different wards of these two hospitals. Microbial load was highest in general ward and lowest in I.C.U. in both the hospitals. *Aspergillus spp.* were the most common fungal isolate in the unit studies, along with *Fusarium* sp, *Mucor* sp, *Verticillium* sp, *Rhizopus* sp, *Penicillium* sp and *Candida* sp. Among various bacteria we isolated *Staphylococcus* sp, *Streptococcus* sp, *Bacillus* sp and *Escherichia coli*. We choose children ward, general ward, causality, I.C.U and burn ward of District hospital as our study area, and to enumerate microbial load in these indoor environments, the air samples were collected three times a day. The highest bacterial population was recorded in afternoon between time 1pm and 2pm compared to morning and evening, ranging from $0.25x10^2$ cfu/m^3 to $7.25x10^2$ cfu/m^3, with the general ward recording the highest bacterial count of $7.25x10^2$ cfu/m^3. The concentration of fungal population in the air of similar wards was recorded high during afternoon sampling between time 1pm and 2pm, with values ranging from $0.12x10^2$ cfu/m^3 to $1.00x10^2$ cfu/m^3.

Key words: *'Hi-Air'* Air Sampler · Hospital · Ward · Airborne microflora · Indoor air

NTRODUCTION

Atmospheric pollution is one of the most drastic problems of our age. This pollution has now reached an advance level those posses potential threat to the health and well being of the population. Different components are found in atmosphere, which enhance or promote the survival of microorganisms in air [1]. Air pollution by micro-biotic entities is a growing menance to human health throughout the world. Various allergic and infectious ailments in man are caused by airborne fungi [2]. The presence of high concentrations of airborne microorganisms within the indoor areola is of increasing concern with respect to many acute diseases, infections, and allergies, and it is an indication of degree of cleanliness of these environments. Indoor environment, of which hospitals is of particular concern, contains different types of microorganisms.

Hospital is an important indoor environment responsible for spread of airborne pathogens [3-5]. It serves as a reservoir of pathogens with individuals infected with number of microorganisms and these are potentially transmitted to other individuals including patients, hospital personnel and even visitors. From the patients, same is subsequently carried over to other patients. Hospital environment is responsible both for indoor and later outdoor community. The hospital environment thus represents a congenial situation where microorganisms and susceptible patients are together indoors. Riley [6] has correctly stated that "the enclosed atmosphere of hospital building and its human occupants constitute an ecological unit".

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A diverse range of microbial population is found in hospital indoor air. The significance of these microbes is debatable in some quarters where as elsewhere it may be considered significant. The estimation of the quantity and types of airborne microorganisms serves as an index for the cleanliness of the environment as well as a profile reveling human health and hospital-acquired infections [7, 8].

The source and spread of organisms inside the hospital are important issues, human related organisms or the body flora, also found in clothing are spread through shedding during human activities [1, 9].

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. Thus evaluation of microbial count, types, and diversity in hospital indoor environments is of growing concern. Hospital- acquired fungal infections are becoming more and more frequent because of the wide-spread and irrational use of broad spectrum antibiotics that are mostly ineffective against fungi [10].

Airborne microflora in hospital rooms was the subject of numerous studies as a potential cause of hospital infections [11, 12]. Most of the studies were performed in intensive care unit, burn ward, general ward, casualty ward, children ward where the risk of infection is greatest [13].

This study was aimed at investigating the concentrations and type of airborne microorganisms in the selected units of the District Hospital and Chaitanya Hospital of Sagar city. Sagar occupies a central position in the country and lies at 23°50'N latitude and 78°48'E longitude, a few miles of northern boundary at the state and is situated over valley tract. District (Tilli) Hospital, a government hospital was chosen for the study due to its high number of patients, visiting both from Sagar and distant villages of Sagar for their treatment. Chaitanya Hospital is one of the private hospitals in Sagar and was chosen for comparative study.

MATERIAL AND METHODS

Study Area: Four wards viz. children ward, general ward, casualty and I.C.U of District hospital and Chaitanya hospital were chosen to comparatively enumerate colony count and to obtain biodiversity of aeromicroflora using settling plate technique. In second set of study, microbial load of indoor air was recorded by *'Hi-Air'* Air Sampler (HiMedia Laboratories Pvt. Limited), during three durations of the day (Morning 9.30-10.30, Afternoon 1.00-2.00 and Evening 4.30-5.30) in five units of District hospital, these include burn ward, I.C.U, casualty, children ward and general ward.

Air Sampling and Microbiological Examination: An adhesive substrate such as a Petri plate containing agar medium is exposed face upwards to the atmosphere to collect particles, settling by gravity. This method is simple, frequently used, sometimes in preference to other aerobiological samplers. It is, however, a passive (non-volumetric) method that does not give information on the volume of air from which the particles have been collected. It also over represents larger particles sampled during the exposure period because of their faster sedimentation rate. Use of settle plates can provide a hint, whether an environment is more or less contaminated with airborne micro-organisms [14]. The horizontal sampling surface provides a measure of deposition per unit area [15]. Most of the air borne fungi except obligate parasites, grow in culture medium. Viable hyphal fragments sporulate helping identification. Using variety of agar based media, is an ancillary measurement for obtaining various forms of microorganisms present in air sample. With this intention, four agar based media plates viz. nutrient agar, malt extract agar, Sabouraud's dextrose agar and Czapex's dox agar were aseptically prepared (composition according to Aneja K.R, 2007 [16]) and were exposed for 5 min at height of 6 feet above the ground in four units of both hospitals. The plates were then closed. Immediately after collection of samples, the Petri plates were taken to the laboratory of microbiology. These exposed Petri plates are incubated in an inverted position at 28±2°C for 3-4 days. Nutrient agar plates were incubated at 36°C for 24-48 hrs. The number of colonies so appeared on exposed plates were counted. The number of microorganisms expressed as CFU/m^3 was estimated using Koch sedimentation method according to Polish Standard PN 89/Z-04008/08, according to which,

$$CFU/m^3 = a.10000/p.\ t.\ 0.2$$

Where:

a-the number of colonies on the Petri plate
p- the surface of the Petri plate
t- the time of Petri plate exposure

Microflora was isolated and sub-cultured in their respective media for further identification. Bacteria were identified using Gram Staining technique and their colony characteristics in various differential and selective media like Eosin Methylene Blue agar Levine (HiMedia

Laboratories Pvt. Limited), Blood agar Base No.2 (HiMedia Laboratories Pvt. Limited), Mannitol salt agar [16, 17]. Lacto-phenol cotton blue was used for staining fungal cultures. Microscopic examination of shape, size and arrangement pattern of spores and other vegetative structures of fungi were employed in their identification [18].

In second set of study, air samples were collected three times a day that is, in the morning, between the hour of 9.30 to 10.30am, in the afternoon 1.00-2.00pm and in the evening 4.30-5.30pm, from five units of District hospital. '*Hi-Air*' Air Sampler System was used for this study. '*Hi-Air*' Air Sampler system (HiMedia Laboratories Pvt. Limited) operate on impaction principle. The air under examination is sucked by the impeller in a 'Torando like' spirally, conical form and the particles contained in it are centrifugally impacted against the inward facing peripheral agar medium strip as the spirally return air escapes around the outer surface of the Tornado. The impeller speed of 4000 rpm is so adjusted that 280 liters of air is sampled every minute. The sampling volume corresponds to 40 liters/ minutes of separation volume i.e. $1/7^{th}$ of the sampling volume based on the mathematical computation in the design principal. The separation volume per unit of time forms the basis of calculating the number of organisms per volume of air being sampled. Sterile plastic strips were aseptically loaded with same agar based media, which were used for settling plate technique. For air sampling, the agar strips were inserted into the slot in the metal cup carefully without touching the agar surface of the strips. After loading the strip, the timer of the control box was set to the sampling time (2 minutes) and the sampler was run by putting the power switch on. After sampling the agar strips were taken out of the sampler's metal cup and the strip was kept back to the wrapper for incubation. After wrapping the strips, the lid of the wrapper is sealed properly to avoid contamination. The strips were then incubated to 5-6 days at 28±2°C temperature. Colonies on the agar stripes were counted with aid of magnifying viewing lens under bright light, after which the number of microorganisms expressed as CFU/m^3 was estimated according to the equation [19] Colony cfu/ m^3= [average colony in even utensil (N) × 1000] ÷[the air flowrate(L/min) × Sampling time(Min)].

$$or\ Cfu/m^3 = \frac{Colonies\ on\ agar\ stripes \times 25}{Sampling\ time\ in\ minutes}$$

RESULTS

After 24 hours of incubation many bacterial colonies appeared in plates, while assurance of fungal colonies was seen only after 2-3 days of incubation on the test media. In settling plate technique, the total airborne microbial population of the four different wards studied in two hospitals varied from ward to wards (Table 1). In the study among the different wards, the children ward, general ward and casualty, were observed to record high

Table 1: Concentration of microflora population in air of four wards in District hospital and Chaitanya Hospital in Sagar city (cfu/m^3)

		Number of Colonies					
		District Hospital			Chaitanya Hospital		
Wards	Name of Media	Fc	Bc	Ac	Fc	Bc	Ac
Children ward	Malt extract agar	Nd	$4.6x10^2$	$0.2x10^2$	$0.2x10^2$	$2.6x10^2$	Nd
	Sabouraud's dextrose agar	Nd	$9.1x10^2$	$0.2x10^2$	Nd	$5.2x10^2$	$0.2x10^2$
	Nutrient agar	Nd	$4.1x10^2$	$0.5x10^2$	$0.2x10^2$	$3.9x10^2$	Nd
	Czapek's dox agar	$0.5x10^2$	$4.1x10^2$	Nd	Nd	$2.0x10^2$	Nd
General ward	Malt extract agar	$0.5x10^2$	$11.7x10^2$	$0.7x10^2$	$0.2x10^2$	$6.2x10^2$	$0.2x10^2$
	Sabouraud's dextrose agar	$0.7x10^2$	$5.2x10^2$	Nd	$0.5x10^2$	$1.5x10^2$	Nd
	Nutrient agar	$0.5x10^2$	$10.4x10^2$	$0.5x10^2$	Nd	$2.0x10^2$	Nd
	Czapek's dox agar	Nd	$7.2x10^2$	Nd	$0.5x10^2$	$3.3x10^2$	Nd
Casualty ward	Malt extract agar	Nd	$9.1x10^2$	Nd	Nd	$3.1x10^2$	Nd
	Sabouraud's dextrose agar	$0.5x10^2$	$7.5x10^2$	$0.2x10^2$	$0.2x10^2$	$5.2x10^2$	Nd
	Nutrient agar	$0.5x10^2$	$4.9x10^2$	Nd	$0.5x10^2$	$3.6x10^2$	$0.2x10^2$
	Czapek's dox agar	Nd	$6.2x10^2$	$0.5x10^2$	Nd	$2.3x10^2$	Nd
I.C.U.	Malt extract agar	$0.2x10^2$	$7.2x10^2$	Nd	$0.2x10^2$	$0.5x10^2$	Nd
	Sabouraud's dextrose agar	Nd	$3.9x10^2$	Nd	Nd	$0.5x10^2$	Nd
	Nutrient agar	Nd	$2.8x10^2$	Nd	$0.5x10^2$	$1.0x10^2$	Nd
	Czapek's dox agar	Nd	$1.5x10^2$	Nd	Nd	$1.3x10^2$	Nd

Fc- Fungal count Bc- Bacterial count Ac- Actinomycetes count Nd- Not detected

Table 2: Concentration of fungal and bacterial population in air of five wards of District hospital in Sagar city (cfu/m^3)

	Morning Sampling time (9.30 to 10.30AM)		Afternoon Sampling time (1.00 to 2.00PM)		Evening Sampling time (4.30 to 5.30PM)	
Wards	Fc	Bc	Fc	Bc	Fc	Bc
Burn ward	$0.25x10^2$	$3.62x10^2$	$0.50x10^2$	$5.00x10^2$	$0.12x10^2$	$4.00x10^2$
Intensive care unit	Nd	$1.37x10^2$	$0.12x10^2$	$0.25x10^2$	Nd	$2.37x10^2$
Casualty ward	$0.37x10^2$	$2.00x10^2$	$0.50x10^2$	$3.75x10^2$	$0.37x10^2$	$2.50x10^2$
Children ward	$0.37x10^2$	$1.62x10^2$	$0.62x10^2$	$3.87x10^2$	$0.50x10^2$	$2.37x10^2$
General ward	$0.50x10^2$	$3.87x10^2$	$1.00x10^2$	$7.25x10^2$	$0.62x10^2$	$4.12x10^2$

Fc- Fungal count Bc- Bacterial count Nd- Not detected

bacterial and fungal counts. General ward of both the hospital had highest microbial load and I.C.U had the least. Comparative analysis of microbial load of these hospitals, reveals less microbial load in indoor units of Chaitanya hospital. Bacteria, fungi and actinomycetes were found in air samples studied. *Aspergillus spp.* were the most common fungal isolate in the unit studies, along with species of *Fusarium*, *Mucor*, *Verticillium*, *Rhizopus*, *Penicillium* and *Candida*. Among various bacteria isolated, we identified *Staphylococcus* sp, *Streptococcus* sp, *Bacillus* sp., *Escherichia coli*.

In our second set of study using '*Hi-air*' Air Sampler, the total airborne microbial population of the five different units studied in district hospital was recorded (Table 2). Significant difference between the number of microorganism sampled in the morning, afternoon and evening occurred in all the units. The highest bacterial population was recorded in afternoon between time 1pm and 2pm compared to morning and evening, ranging from $0.25x10^2$ cfu/^{3} to $7.25x10^2$ with the general ward recording the highest bacterial count of $7.25x10^2$. The concentration of fungal population in the air of similar wards was recorded high during afternoon sampling between time 1pm and 2pm, with values ranging from $0.12x10^2$ cfu/m^3 to $1.00x10^2$ cfu/m^3. There was negligible presence of actinomycetes.

DISCUSSION

Airborne fungi are not uncommon and for most individuals breathing typical ambient concentrations of airborne fungi results in no adverse effects on health due to healthy immune system. However, hospitalized patients with immune suppression are susceptible to infections from naturally occurring airborne fungi that can grow at body temperatures. The incidence of infections caused by fungi that were once considered only saprophytic has raised dramatically in recent years, for example, hospital mortality caused by invasive aspergillosis [20]. Aspergillosis is acquired by inhalation of airborne dust particles that carry the spores. Pneumonia develops and the fungus disseminates through the blood stream to other organs. Mortality rates have been reported as high as 95% in bone marrow transplant patients; 13-80% in leukemia patients; and 8-30% in kidney transplant patients. One researcher calculated that about 9% of reported hospital infections between 1986 and 1990 were caused by fungi [21].

The quantitative study of different hospital units showed that, the General ward recorded the highest airborne bacterial and fungal population, while the least airborne bacterial and fungal population was recorded in Intensive care units of both the hospitals. The high microbial counts recorded for the District hospital as compared to the private hospital (Chaitanya Hospital), could be due to the subsidized rate of public hospital so as to accommodate more people, compared to private hospital, where high fees are charged and are not within the poor people in the society. There is more visit of patients as well as visitors in general ward as compared to I.C.U, which is also one of the factors for high microbial count in general wards. Personal belongings, food and fruits are recognized as source of hospital contamination. Microbial load in hospital indoor air is highly influenced by the number of occupants, their activity and the ventilation. Shedding of microorganisms from the skin surface and the respiratory tract of the occupants increases microbial load [14].

The microbial isolates characterized and identified included four bacterial and six fungal isolates, they include, *Staphylococcus* sp, *Streptococcus* sp, *Bacillus* sp., *Escherichia coli* for bacterial isolates, while the fungal isolates includes *Fusarium* sp, *Mucor* sp, *Verticillium* sp, *Rhizopus* sp, *Penicillium* sp and *Candida* sp. Few actinomycetes were also found in air samples. Air is not a suitable medium for growth of the pathogenic bacteria, any pathogen that air borne, must have originated from a source such as humans,

food or water. Isolation of *E. coli* might be due to fecal contamination and are used as indicator organisms, indicating the possibility of occurrence of different disease agents especially in immunocompromised persons [22]. *E. coli* is commonly associated with water also and its presence in the environment reflects the degree of purity of the water used for various purposes e.g. floor cleaning and other activities in hospital wards. The aerobic actinomyces are ubiquitous. The pathogenic aerobic actinomycetes usually cause a chronic exudative inflammatory infection but may occasionally cause an acute, necrotizing pyogenic infection. *Aspergillus* spp can be transmitted from patients or the environment [23]. Even though it is harmless for healthy people, *Aspergillus* may be dangerous for patients of risk group, included those treated in intensive care units. The tiny spores readily invade upper and lower airways and may produce lung aspergillosis in risk group patients [24]. Exogenous infection of Candida (Candidiasis) is also possible and it is potential source of infection, especially in risk group patients. *Mucor* and *Rhizopus* may invade patients with inhaled air as well as through the equipments used in diagnostic and care of patients.

It follows that concentrated population and weak ventilation are two important factors for the content of airborne microbes in indoor environment. The decrease of number of fungi and bacteria in the morning and evening might have been caused by ventilation of the rooms and /or lower number of people present in the rooms by day.

CONCLUSION

Indoor air monitoring focused on the presence of fungi and bacteria are important procedures and such procedures should be routine in hospitals. Efforts are needed to improve hospital hygienic environment and it is recommended to raise the awareness and educational status of medical workers to reduce the hazards of air-borne transmission of such potentially pathogenic microorganisms.

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