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STUDY OF ISOLATION OF FUNGAL SPORES FROM THE HOSPITAL AIR SPECIMEN

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ABSTRACT.

Fungi are everywhere in our surroundings, but only a few people truly recognize the profound connection between fungi and our lives. Mycotic diseases affecting humans are an emerging public health concern, receiving increasing attention from health authorities. Certain human activities and changes in behavior contribute to varying susceptibilities among different populations. Within hospitals, nosocomial infections occur through three primary environmental routes: Air, surface contact, and water. This study presents the findings of a survey conducted to monitor fungi in specific areas of a KHYBER HOSPITAL SRINAGAR. The air samples collected in the hospital primarily contained Aspergillus spp and other species such as Dematiaceous fungi, Trichosporon, Absidia, and Rhizopus.

INTRODUCTION

The rising occurrence of healthcare-associated infections, particularly opportunistic fungal infections, has garnered significant attention in recent years (1, 2). Hospital acquired invasive fungal infections have risen, threatening patients' lives (2). The transmission routes for these infections include airborne transmission, contact transmission, standard vehicles, medical devices, and instrumentation (3). Airborne transmission, in particular, is a primary concern in hospitals, and preventing the spread of airborne pathogens is crucial for controlling infections (3, 4). While effective environmental monitoring can help reduce hospital infection rates, the lack of welldeveloped methodologies for monitoring changes in airborne pathogens hampers managing and controlling hospital infections (5). Pathogenic fungi are responsible for causing diseases in humans and other organisms. Fungi are omnipresent in our environment. The mortality rate due to fungal infections is comparable to that of malaria or tuberculosis, estimated to affect around 1,350,000 patients annually (6). With their increasing morbidity and mortality rates, fungal infections acquired in hospitals have gained significant importance in recent years (7, 8). Nosocomial infections, or hospital-acquired infections, are a leading cause of death worldwide, predominantly affecting immunocompromised patients. This study presents the findings of environmental surveillance of fungi conducted in specific areas of Khyber hospital srinagar. The objective was to investigate the prevalence of nosocomial infections and determine the reported cases of fungal infections in the hospital within or from the community—air samples from significant hospital areas for this surveillance. Mycotic diseases in humans present an emerging public health challenge,

drawing increasing attention from health authorities (9, 10). Fungi have three significant adverse effects on human health: inflammatory, allergic, and toxic. Airborne transmission is the most common means of exerting these effects on humans (11). One of the most significant challenges in controlling these diseases is the constant exposure of patients to high concentrations of fungal spores within hospital wards. Therefore, assessing the distribution of airborne fungi in hospitals, mainly through air conditioning surveillance, is crucial. Aspergillus spp. are significant fungal pathogens associated with respiratory diseases (11). The severity of Aspergillus infections varies depending on the host'simmune status, ranging from hypersensitivity reactions to fatal invasive pulmonary disease (12). Involve a complex interaction between the respiratory epithelium and the host response in the presence of inhaled spores (10).
Fungi are eukaryotic organisms that externally digest food and absorb nutrients through cell walls.Most fungi reproduce through spores and consist of hyphae, microscopic tubular cells that form their body (thallus). Like animals, fungi are heterotrophs and obtain carbon and energy from otherorganisms (13, 14).

Mycology is the branch of biology dedicated to the systematic study of fungi, including their genetic and biochemical properties, taxonomy, and uses for humans in medicine, food, religious practices, and associated dangers such as poisoning and infection (15). Mycology emerged as a systematic science after the invention of the microscope in the 17th century. While fungal spores were by Giambattista della Porta in 1588, Pier Antonio Micheli's 1729 work "Nova plantarum genera" is considered seminal in developing mycology. Micheli observed spores and demonstrated that, under appropriate conditions, they could grow into the same species of fungi from which they originated. Building upon Carl Linnaeus' binomial nomenclature system introduced in "Species Plantarum" (1753), Christian Hendrik Persoon

Some Fungal Human Pathogens

When it comes to discussing fungal diseases, the most practical approach to classification is to group them based on the specific type of infection they cause:

1.Superficial infections Fungal diseases are caused by organisms that target the skin or its appendages (such as nails, feathers, and hair), to as dermatophytosis. These infections include ringworm, jock itch, and athlete's foot. The responsible fungi for these conditions are known as dermatophytes.

2.Systemic infection Systemic fungal diseases by infections that deeply infiltrate tissues, affecting vital organs and the nervous system. These conditions can range from life-threatening to chronic. Typically, entry into the body occurs through inhalation of spores or open wounds. Once inside, the fungus can spread throughout the body via the bloodstream or respiratory system, leading to additional infections in internal organs. These fungi are typically saprotrophic, thriving in soil environments.

1. **Intermediate infection**, There is a category of fungal infection that is an infections occur beneaththe skin but remain localized without spreading extensively.
   Fungal diseases pose more significant challenges for treatment compared to bacterial infections. Typically, topical and oral treatments are required and must be for an extended period. However, even with diligent treatment, success in completely eradicating the fungus may be limited. Many fungal infections tend to become chronic and can recur even after initially successful treatment.

One of the reasons treating fungal diseases is difficult is due to the similarity between fungal cells and human cells, as both are eukaryotes. When specific chemicals target fungal cells, there is a risk of unintended damage to our cells due to these similarities. Surveillance becomes crucial to control and manage infections within specialized hospital units effectively.

This study uses an Air Sampler device to collect air samples from 25 different areas within the hospital.

FUNGAL SPORES IN AIR

Fungal spores are commonly spread through the AirAir, allowing them to be present in the AirAir we breathe. However, the airborne environment poses challenges for fungi due to low water availability and high radiation levels. Consequently, most fungal spores cannot survive for extended periods in the Air without a source of energy. Nevertheless, the spores that survive have developed specific mechanisms to protect themselves from drying out and being damaged by radiation.

The spores of common airborne fungi possess robust, melanized walls. These walls typically contain complex carbohydrates that are both hydrophobic and waxy. The wall's hydrophobic nature allows for water loss control while still allowing water uptake before germination. Therefore, the survival of spores in the Air depends on finding a delicate balance between preventing water loss and maintaining metabolic activity. The cytoplasm of airborne spores may contain higher concentrations of compatible solutes like glycerol and other polyols, enabling sustained metabolism in limited water availability conditions. Glycerol in the cytoplasm allows water to be lost from the cell without severe consequences when in moist conditions.

Various molecules, including melanin, are present in spores and reduce radiation penetration, particularly in the UV range. Melanin, found in the spore walls, is the primary barrier against UV radiation, additionally in spores. As a result, the energy from radiation into heat can move away from the spore in the Air.

In most cases, inhaling fungal spores does not affect humans. The spores settle on the moist surfaces of the respiratory tract lining and through mucus. The immune system neutralizes any remaining spores. However, a few fungi can evade the immune response and cause respiratory diseases. nature allows for water loss control while still allowing water uptake before germination. Therefore, the survival of spores in the AirAir depends on finding a delicate balance between preventing water loss and maintaining metabolic activity. The cytoplasm of airborne spores may con tain higher concentrations of compatible solutes like glycerol and other polyols, enabling sustained metabolism in limited water availability conditions. Glycerol in the cytoplasm allows water to be lost from the cell without severe consequences when in moist conditions.

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MATERIAL AND METHODS

The current study was at IVY Hospital [POLO LABS], Mohali. The objective was to collect air samples from various wards within the hospital and the surrounding outdoor environment. Therewere 25 areas within the hospital for air sampling, and three times to ensure accurate and reliableresults.

Materials used

The materials used in the present study are in the following table. The media and chemicals used from Hi Media TM Laboratories Pvt. Ltd. Mumbai, India

Media Preparation Sabouraud Dextrose Agar

Sabouraud Dextrose Agar (SDA) is a selective medium commonly employed for the isolation of dermatophytes, as well as other fungi and yeasts. The acidic pH of this medium, which is approximately 5.0, restricts bacterial growth while facilitating the development of yeasts and most filamentous fungi. Additionally, antibacterial agents can be incorporated to enhance the antibacterial properties of the medium.

Principle:

The SDA medium contains an enzymatic digest of casein and animal tissues that offer a rich source of amino acids and nitrogenous compounds essential for the growth of fungi and yeasts. It also includes dextrose in high concentration as a fermentable carbohydrate to serve as a carbon and energy source. Agar as a solidifying agent.

Additionally, the medium may consist of broad-spectrum antibiotics such as Chloramphenicol and tetracycline, which can inhibit the growth of a wide range of grampositive and gram-negative bacteria. Gentamicin can also to further restrict the growth of gram-negative bacteria (55). Media Composition:

Dissolve 65 grams of the medium in 1000 ml of distilled water. Heat the mixture to boiling until medium. Sterilize the medium by autoclaving at 15 lbs (121°C) for 15 minutes.

Once samples were collected and incubated adequately, fungal colonies displayed characteristic color and morphology—filamentous colonies of different colors in the case of mold growth (56).

Potato Dextrose Agar Potato Dextrose Agar is a general-purpose medium that stimulates sporulation and pigmentation, making it helpful in cultivating and differentiating pathogenic and nonpathogenic fungi. Bacterial growth could interfere with the recovery of yeasts and molds.

Principle:

Potato infusion and dextrose provide abundant nourishment for fungal growth—Potato Dextrose Agar to cultivate yeasts and molds. The injection from potatoes offers essential nutrients that promote sporulation

and pigmentation production. The medium's acidic pH creates a favorable environment for fungal growth while inhibiting the growth of bacteria that could hinder the recovery of yeasts and molds.

Media Composition:

Ingredients	g / liter
Potatoes, infusion from	200.000
Dextrose	20.000
Agar	15.000
Final pH (at 25°C)	5.6 ± 0.2

To prepare the medium, dissolve 39 grams in 1000 ml of distilled water and heat it to boiling until the medium is. Sterilize the medium by autoclaving at 15 lbs pressure
(121°C) for 15 minutes. Ensure thorough mixing before dispensing; in specific cases where a pH of 3.5, acidify the medium using sterile 10% tartaric acid or lactic acid.
It is important not to heat the medium after adding the acid.
After collecting air samples and incubating the plates, filamentous colonies of various colors indicate the growth of molds (57).

Air Sampler

Working principle:

The Air petri sampling system utilizes the sieve impactor principles described initially by Andersen (58, 59). This system draws Air through a perforated plate, which directs the resulting air stream and any particles on the surface of a standard Petri dish containing nutrient agar or other desired medium. Following a cycle, the Petri dish and the resulting colonies are counted and expressed as colony-forming units (cfu/m).
The system comprises a container that holds a petri dish with the chosen medium and a perforated cover with a pre-determined hole size. A vacuum pump draws a known volume of Air through the body, and ythe particles, including microorganisms are deposited on the agar medium plate.

RESULTS

Aspergillus spp., including Aspergillus fumigatus, Aspergillus, and Aspergillus niger, along with Rhizopus spp., dematiaceous fungi, Trichosporon spp., and clusters of Gram-positive cocci, were identified in the air samples collected from the hospital (Table 4). Among these, the most frequently encountered fungus was Aspergillus spp.

Table : List of Hospital Air Mycoflora

S.NO.	**Ward**	**Bacteria** **Isolated**	**Molds** **Isolated**
1.	Outdoor environment	Nil	*Aspergillus spp,* *Aspergillus niger,* *Aspergillus nidulans,Rhizopusspp,* *Trichosporon, Dematiaceous fungi, Absidia*
2.	Private wards		
	A2	Gram Positive cocci (in clusters)	*Aspergillus niger,* *Aspergillus*
			fumigatus,

	A3	Gram Positive cocci (in clusters)	*Aspergillus spp,* *Aspergillus niger*
	A4	Nil	*Aspergillus spp,*
			Aspergillus niger
3.	Operation theaters	Nil	*Aspergillus niger, Aspergillus fumigates*
4.	Intensive Care Units	Gram Positive cocci (in clusters)	*Aspergillus spp,* *Aspergillus niger, Aspergillus fumigates*

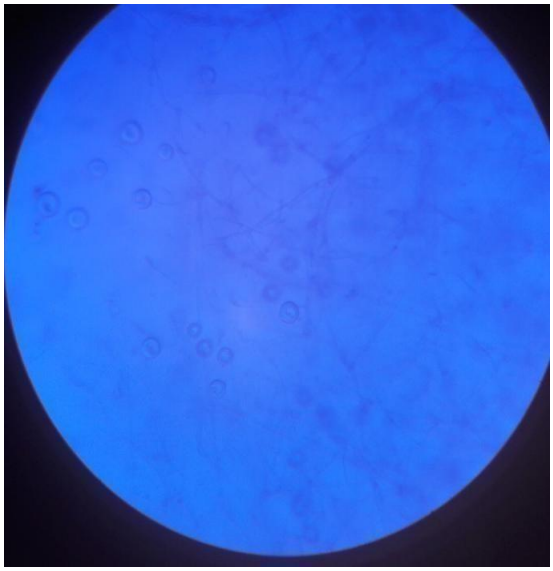


Figure 10: *Aspergillus*

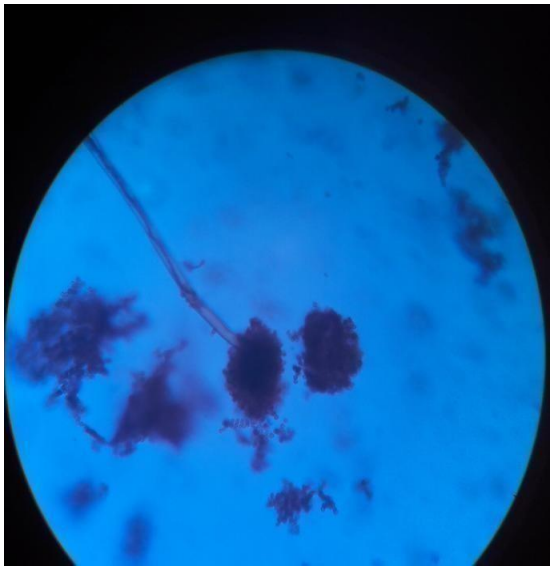
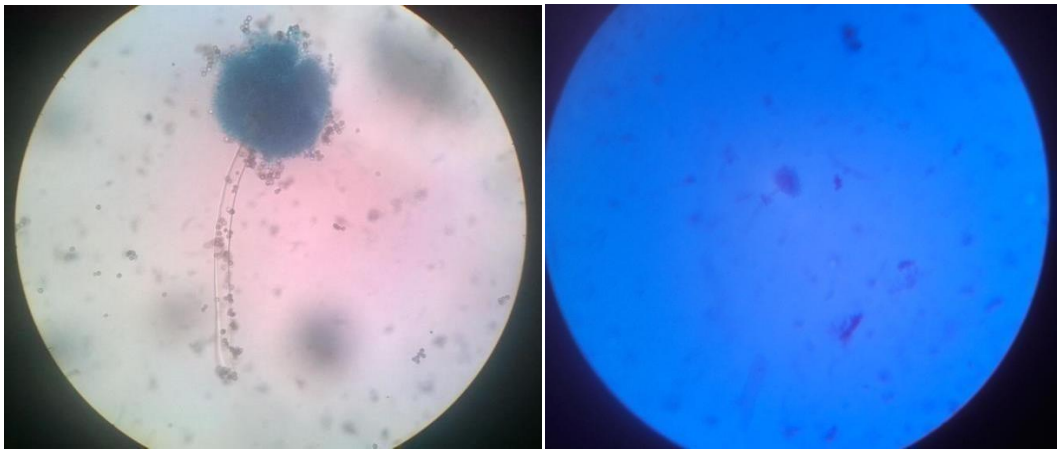


Figure 11: *Dematiaceous fungi*

Figure 12: *Aspergillus niger* **Figure 13:** *Aspergillus fumigatus*



DISCUSSION

This study investigated the influence of two factors, the type of room and the time of sampling, individually or combined, on the microbial rate in the indoor air of a hospital. The findings revealed that the outdoor hospital environment exhibited a higher level of contamination than airborne fungi in the indoor AirAir. Additionally, the fungal load in private wards was relatively higher than in intensive care units and operating theaters. These results indicate that the type of ward significantly affects the rate of airborne fungi in the indoor AirAir of the hospital. Considering the type of room, such as intensive care units, operation theaters, or neonatal wards, as a factor influencing the indoor rate of airborne fungi, different cleanliness and disinfection strategies had a significant impact. That might increase fungal rates in patient rooms, as many people, including patients, visitors, and personnel, frequently occupy these areas, leading to an increased indoor rate of airborne fungi. Many patients, visitors, and personnel contribute to higher microbial rates, particularly in the afternoon when activity levels peak. Some private wards and intensive care units also exhibited contamination of Gram-positive cocci in clusters in the Air.The exchange of indoor and outdoor AirAir increases the microbial rate due to the introduction of microorganisms from outside the hospital through open windows and doorsat the main entrance.

The airborne fungal species identified in this study have been in several previous studies that employed different isolation and identification procedures. These fungal pathogens are known to cause infections in immunocompromised patients. The permissible reference for fungal spores in the hospital environment is 25 CFU/m3; the fungal spore count in our hospital was within this limit. The predominantly isolated fungus was Aspergillus spp., including Aspergillus fumigatus, Aspergillus niger, Aspergillus, along with Rhizopus spp., Trichosporon, dematiaceous fungi, and Absidia, which were found both in the outdoor and indoor environments of the hospital.

CONCLUSION

Mycotic infections have become a significant global public health issue in the modern era.The presence of fungal contamination in healthcare facilities has been the subject of numerous studies. During our visit to the hospital, five fungal genera, namely Aspergillus,Dematiaceous fungi, Absidia, Trichosporon, and Rhizopus, were isolated and identified from both the outdoor and indoor air samples. The main objective of this study was to assess the fungal load and diversity in the hospital environment and its potential impact onpatients.

Our findings indicate that the outdoor environment and certain private wards exhibited a higher level of contamination with airborne fungi in the indoor AirAir. However, fungal contamination in the operating rooms was shallow. It is worth noting that the fungal spore count in our hospital remained within the permissible reference range for fungal spores in the hospital environment (CFU/m3).

It is to adhere to good infection control practices, including proper hand hygiene, adequate sterilization measures, and air filters in patient rooms. These measures will significantly contribute to maintaining a safe and hygienic environment in the hospital, thereby reducingthe risk of fungalrelated diseases.

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