ISOLATION OF FUNGAL SPORES FROM THE HOSPITAL AIR SPECIMEN

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Abstract

Despite the fact that fungi are all around us, few people are aware of how closely we are connected to them. Men's myccoti illnesses are a rising public health issue that receives support from the relevant authorities. Special human activities and changes in human behavior are responsible for different susceptibilities in individual populations Three mainenvironmental routes air, surface contact, and water are used in hospitals to transmit nosocomial diseases. The findings of environmental fungus surveillance in particular Tertiary Care Hospital (Mohali) locations are presented in this study. The majority of the Aspergillus species, as well as other species like Dematiaceous fungi, Trichosporon, Absidia, and Rhizopus, were found in the hospital's air samples.

KEY WORDS,*fungi, myccotiC, nosocomial,* Aspergillus spp, Mycology

INTRODUCTION

The rise in healthcare-associated infections has drawn considerable attention in recent years (1, 2). Invasive fungal infections are becomingmore common in hospitals, endangering the lives of the patients who contract them (2). Airborne, contact, common transportation, medical gadgets, and instruments are some of the transmission channels for infections(3).The prevention of airborne pathogens is crucialfor the management of hospital infections since airborne infection is regarded as a key channel of transmission in hospitals(3,4).Although the management and control of hospital infections are limited by the lack of developed approaches for monitoring changes in airborne pathogens, effective environmental monitoring could aid in lowering infection rates at hospitals (5). fungus that can infect people or other organisms with sickness are called pathogenic fungus. Our environment is constantly inhabited by fungi. The death ratefrom fungal illnesses is comparable to that from malaria or tuberculosis and is predicted toreach over 1,350,000 individuals annually (6). Fungal infections with hospital origin are becoming more significant in recent years because to their steady rise into high rates of mobility and mortality (7), Man-caused mycotic illnesses are an emerging public health issue that is receiving increasing attention from the relevant authorities. Three significant unfavourable consequences of fungi (9, 10) on human health are inflammatory, allergic, andtoxic. Humans are typically exposed to the first two categories of effects via airborne means(11). The fact that patients are frequently exposed to large levels of fungal spores in hospital

wards is the biggest obstacle to the control of these infections. As a result, determining thedistribution of airborne fungus in hospitals is crucial, especially for air quality monitoring. Significant fungal infections associated with airway illness include Aspergillus spp. (11). The kind of aspergillosis depends on the host immunological condition and can range from a catastrophic invasive lung disease to a hypersensitive reaction (12). The respiratory epithelium and the host response interact intricately in the presence of inhaled spores to produce the range of pulmonary illness linked to Aspergillus spp, (10).

An eukaryote called a fungus digests food outside and takes nutrients straight into its cell walls. The majority of fungus produce spores and have a body (thallus) made of tiny tubularcells known as hyphae. Since fungi are heterotrophs, they, like animals, rely on other speciesfor their carbon and energy (13, 14).

Mycology is the branch of biology that deals with the systematic study of fungi, including their genetic and biochemical properties, taxonomy, human uses as a source of food, medicine, and psychoactive substances used for religious purposes, as well as their risks, such as infection or poisoning (15). Mycology is a relatively new field of study that becamesystematic in the 17th century with the invention of the microscope. The publication of PierAntonio Micheli's 1729 book Nova plantarum genera is regarded as the fundamental work in the development of mycology, even though Giovanni Battista della Porta first noticed fungal spores in 1588. Micheli not only observed spores but also demonstrated how they could be made to develop into the same species of fungi from which they came in the right circumstances. Increasing the usage.

Some Fungal Human Pathogens

In discussing fungal diseases, the most convenient way of classifying them is to categorize them according to the type of infection that has occurred:

1. Superficial infections are caused by fungi that attack the skin or its appendages (nail, feathers and hair). Some examples of these infections include ringworms, jock-itch andathlete's foot. These fungi are known as dermatophytes.
2. Systemic infection are diseases that occur deep within the tissues, involving vital organsand/or the nervous system, and which may be fatal, but may also be chronic. Entry intothe body is usually through inhalation of spores or open wounds. Blood circulation or respiratory system may then transmit fungus throughout body and additional infection of internal organ may occur. These fungi are usually saprotrophic fungi, growing in thesoil.
3. Intermediate infection, is sometimes also recognize and is intermediate between the twojust discussed. The infection will occur below the skin, but will remains localized.

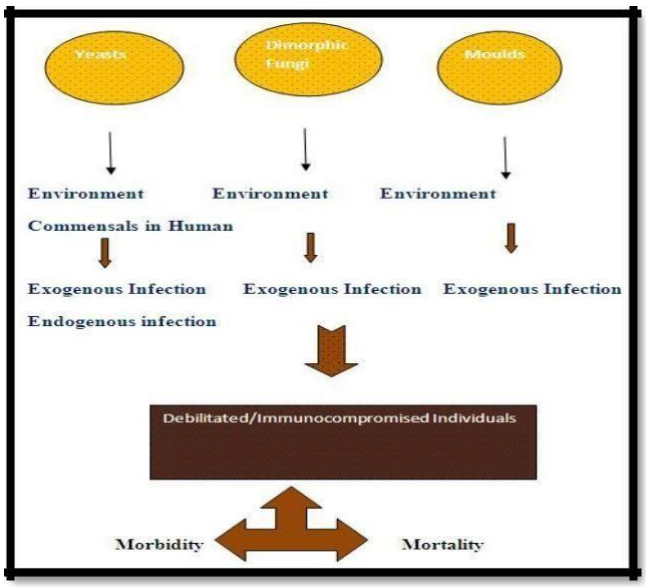


Figure1: Flow chart representing source and consequence for IFI's

Unlike bacterial diseases, fungal diseases are more difficult to treat. Often topical and oral treatments are long term and may only be partially successful in controlling the fungus. Many types of these infections can be chronic and reoccur even after successful treatment. The difficulty in treating fungal diseases is that fungi are eukaryotes as human cells and when the fungal cells are targeted with the help of some chemicals, our own cells also get damaged because of the similarities (17).

So, the Surveillance is the core method used to control and manage infections in special units of hospitals (18). In this study, I have utilized an Air Sampler device and collected airsamples from25 different areas of the hospital for monitoring the surveillance of the hospital environment and cultured fungus on SDA media plates which were incubated at37˚C.

MATERIAL AND METHODS

The present study was undertaken in the IVY Hospital [POLO LABS], Mohali. Air sampling was performed in the different wards of the hospital and also in the outer environment of the hospital. Air sampling was done in 25 areas of the hospital and the samples were taken3 times in each area to interpret the faultless results.

Materials Used

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

Table2: *List of materials used in the project*

S.No. Material used Type Purpose
1. Media Sabouraud Dextrose Agar Sample collection
Potato Dextrose Agar Slide culture
2. Stainingre agents Crystalviolet Gram'sstain (Primarystain)
Gram'siodine Gram'sstain (Mordant)
Acetone Gram'sstain (Decolourizer)
Safranin Gram'sstain (Counterstain)
Lactophenol cotton blue For LCB preparation (Fungal identification)
3.Instruments and Air Sampler For collection of Air samples Equipment'sSafety Cabinet For culturing
Microscope For identification
Microbiology Incubator Incubation
Autoclave Media preparation.
4. Glassware Conicalfl asks Media preparation
Measuring cylinder Media preparation
Petriplates Media preparation, Slide culture
Glass slides Gram staining, LPCB, Slide culture

Media Preparation Sabouraud Dextrose Agar

Sabouraud Dextrose Agar Most fungi of medical importance grow well on Sabouraud dextrose agar. It is an acid pH medium that allows the growth of dermatophytes, Candida and other fungi and acidophilic bacteria. It contains: Peptone (mycological) 5.0 g 20.0 g Dextrose Agar Distilled water 7.5 8 500 ml pH 5.5 It is available in dehydrated powdered form or tablet form from manufacturers of culture media. The powdered form is used at a concentration of 65 g per liter of distilled water, and the tablets 1 tablet in 5 ml of distilled water. The Sabouraud medium can be made more selective when used with certain antibiotics in order to isolate pathogenic fungi from material containing large numbers of other fungi and bacteria: 0.5 g cycloheximide in 1 liter of medium inhibits Cryptococcus neoformans and Asper gillus

fumigatus: 20,000 units penicillin and 40,000 units streptomycin in 1 liter of medium inhibits Actinomyces bov is and Nocardia aesteroides,


air sampler

Stability of the Reagents

All the reagents are stable at room temperature (25°C +5°C).

A. Smear preparation

1. Take a grease-free dry slide and make an oval- shaped mark at the center by using a glass marker.
2. Sterilize the inoculating (nichrome) loop on the flame of a bunsen burner.
3. Transfer a loopful of culture (or specimen) by the sterile nichrome loop and make a smear in the pre marked area on the slide (smear should not be very thin as well as verythick).
4. Allow the smear to dry in the air.
5. Fix the dry smear by passing the slide 3 to 4 times through the flame quickly with the smear side facing up.

B. Gram staining

1. Place the slide on the staining glass rods.
2. Cover the smear with crystal violet stain and leave for 1 minute.
3. Wash carefully under running tap water. 4 Flood the smear with Gram's iodine solutionand wait for one minute.
4. Drain off the iodine.

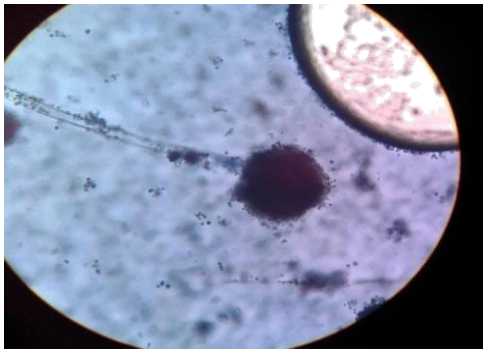
5. Decolorize the smear with alcohol-acetone (or rectified spirit) for 20 to 30 seconds.Continue till purple stain just stops coming on the slide.
6. Gently wash the slide under running tap water and drain completely.
7. Counterstain the smear with safranin for 10 seconds or with dilute (1:20) basic fuchsinfor about 1 minute.
8. Drain the staining solution and allow the stained smear to dry in air (or dry it carefullyby using a blotting paper).
9. First observe for uniform stained area under low power objective, afterwards under highpower objective, and finally under oil immersion objective.

Lactophenol cotton blue (LPCB):

Lactophenol Cotton Blue (LCB) Reagent

This reagent can also be used for staining as well as for wet mounting of fungi. Lactic acidpreserves the fungal structure and clears the tissue while phenol acts as a disinfectant. The LCB reagent is prepared as follows:

1. Lactic acid: 20 g
2. Phenol: 20 g
3. Glycerine: 40 ml
4. Distilled water: 20 ml



Aspergillusin LPCB mount

RESULTS

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After incubating the plates for 2-3 weeks at 37˚C identification of the fungal species was done on basis of the colony morphology, slide culturing and LPCB preparation. The CFU count perm number of colonies on all plates was only1-2 was ≤25 CFU/m as the

Figure: *Aspergillus nidulans*

Figure:*Aspergillusniger*

Figure:*Absidia*

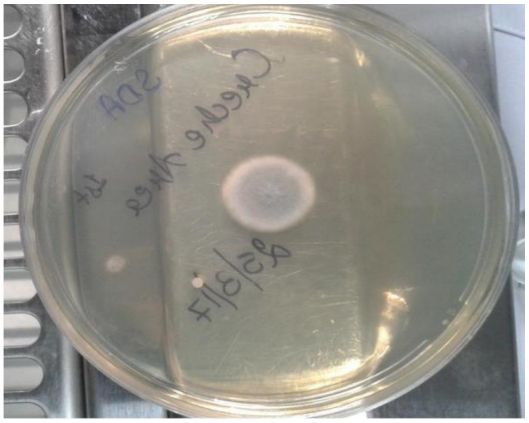

Figure:*Dematiaceousfungi*

The air samples in the hospital yielded Aspergillus spp, Aspergillus fumigatus, Aspergillus nidulans, Aspergillusniger, Rhizopusspp, dematiaceous fungi, Trichosporonspp and Grampositive cocci presentin clusters (Table 4). The most common fungus isolated was Aspergillus spp.

Table 4: List of Hospital Air Mycoflora

S.NO.	Ward	Bacteria Isolated	Moulds Isolated
1	Outdoor environment	Nil	*Aspergillusspp,*
			Aspergillusniger,
			Aspergillusnidulans,
			Rhizopusspp,
			Trichosporon,
			Dematiaceousfungi,
			Absidia
2	Private wards		
	A2	Gram Positive	*Aspergillusniger,*
		Cocci (in clusters)	*Aspergillusfumigatus,*
	A3	Gram Positive	*Aspergillusspp,*
		Cocci (in clusters)	*Aspergillusniger*
	A4	Nil	*Aspergillusspp,*
3	Operation theaters	Nil	*Aspergillusniger,*
			Aspergillusfumigates
4	Intensive Care Units	Gram Positive	*Aspergillusspp,*
		Cocci (in clusters)	*Aspergillusniger,*
			Aspergillusfumigates

The LPCB mount were prepared and have shown the morphological structures of the fungalspecies under 40x objective of the microscope (Fig.9, 10,11,12).

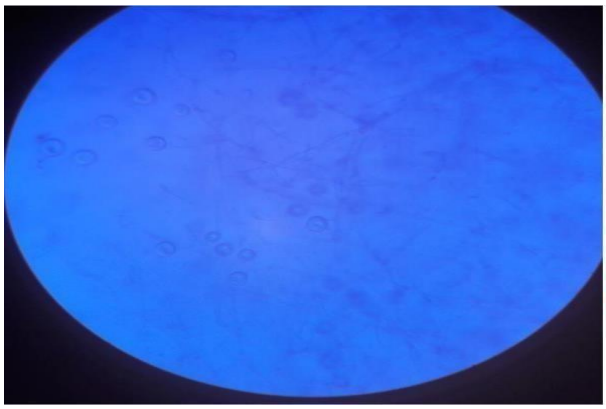

Figure9: Aspergillus nidulans

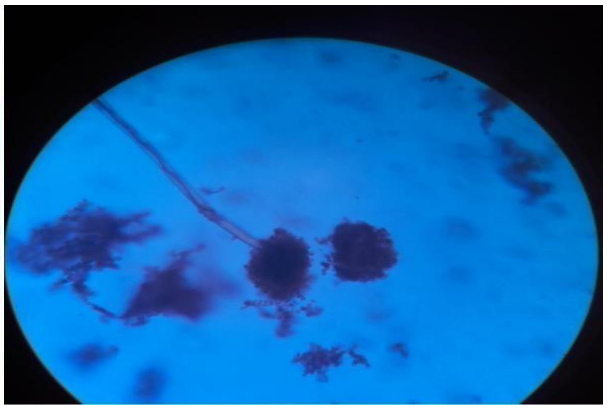

Figure10:Dematiaceousfungi

Discussion

The two criteria that were examined in this study, the kind of room and the time of sampling, either separately or together, were discovered to have an impact on the microbiological rate in indoor air of hospital infections. The findings of this investigation demonstrated that indoor airborne fungus were less contaminated than the outdoor hospital environment. Additionally, the private wards' fungal loads were higher than those of the intensive care units and operating rooms.

Conclusion

Mycotic infections are a significant public health issue due to their global prevalence. Numerous research have examined the presence of fungi in healthcare settings. At the time of the hospital visit, five fungal genera were isolated and identified from indoor and outdoor air: Aspergillus, Dematiaceous fungi, Absidia, Trichosporon, and Rhizopus. Determining the fungal load and variety issues in the hospital patient environment is the study's main goal.

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