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Study of fungal load in wards, departments and doctors' equipments in a tertiary care hospital and evaluation of 3 new mounting solutions for fungal micromorphological study

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Abstract
Fungi are very important causes of nosocomial infections. Fungal infections of hospital origin are gaining importance in recently due to their progressive increase and high mortality and morbidity with which they are associated. Unpleasant hospital IAQ may lead to hospital-acquired fungal infections, sick hospital syndrome, and various occupational risks. Numerous bacteria and fungi have been documented on mobile phone screens of doctors and nurses. Lactophenol cotton blue is an age old mounting medium for studying fungal colony micromorphology but has limitations like high cost. In our study we assessed fungal bioload in hospital environments like tables, doctors' stethoscopes, mobile phone screens, and also found out 3 mountig fluids which are cheaper alternatives to Lactophenol cotton blue. Air was sampled by setle plate technique and culture of swab was done on Sabouraud's dextrose agar. Load of fungi like Aspergillus spp. and Penicillium was very high in these objects.

Keywords: fungal bioload, LPCB, Environment, SDA

1. Introduction
Pathogenic fungi are fungi that cause disease in human or other organism, among the 1.5 million different types of fungi that are present everywhere [1]. Fungal infections of hospital origin are gaining importance in recent times due to their progressive increase and high rates of mortality and morbidity with which they are associated [2]. Many of these infections are endogenous in nature, but others can be acquired by exogenous routes, by the hands of healthcare workers, contaminated infusion products and biomaterials and environmental sources [3]. In fact, bacteria and fungi abound on surfaces of stethoscope diaphragms and add to the burden of nosocomial infections [4]. In hospital facilities, Indoor Air Quality (IAQ) is a critical factor in preventing infections, and unpleasant hospital IAQ may lead to hospital-acquired infections, sick hospital syndrome, and various occupational risks [5]. Numerous bacteria and fungi have been documented on mobile phone screens of doctors and nurses [6]. Lactophenol cotton blue is a mounting solution that is commonly used to study microscopic morphology of fungal colonies, the disadvantage of LPCB being its high cost [7]. Keeping all these things in mind, our study was planned to study fungal colonisation of hospital air, tables, mobile phone screens and stethoscopes of our hospital.

2. Materials and methods
2.1. Type of study
This was a laboratory based observational study, carried out in the Department of Microbiology of the institutes as a part of summer training research cum dissertation over a period of 2 months from March 2017 to April 2017. Samples were collected from different wards of this hospital. Isolated fungi, were examined microscopically by making three mounting fluids: MAK (Methylene blue 0.14% solution 2 ml, Glacial acetic acid 10 ml, 10% KOH 88 ml), GAW (Giemsa working solution 2 ml, Glacial acetic acid 10 ml, Deionised water 88 ml), and CVEH (Crystal violet-0.5gm,70% Ethanol-80ml, 5 % H2SO4-20ml). These three mounting fluids were compared with LPCB.

2.2. Method of Air mycoflora collection
Settle plate method was used to trap air- borne fungal species in the atmosphere of the hospital rooms of 13 different wards at AIIMS Hospital, during the period of March 2017 to April 2017.
Previously prepared Sabouraud's Dextrose agar (SDA, Hi Media labs, India) containing Petri plates, a medium suitable for growth of fungi, was exposed at different corners of the wards for one hour, 1 meter away from the wall, and 1 metre above the floor. This allowed mold spores and fragments to settle onto agar media by gravity.

2.3. Methods of stethoscope, mobile, and table mycoflora collection.
Each swab sticks were dipped in 0.9% saline or sterile peptone water and rubbed over the diaphragm of the stethoscopes, screen of mobiles and surface of tables and subsequently inoculated on SDA slants respectively.

Isolation and identification
Samples were inoculated on SDA slants or plates. The tubes and plates were incubated at 37 °C aerobically for upto 3 weeks.
The obtained colonies were examined under microscope by preparing LPCB (Lacto phenol cotton blue), MAK (methylene, acetic acid, KOH), GAW (Giemsa solution, acetic acid, water), CvEH (crystal violet, ethanol, H2SO4) mounts.

2.4 Preparation of mounting solution for fungus identification

Solution I

- MAK (Methylene, acetic acid, KOH).
  Methylene blue- 2ml
  Acetic acid -10ml
  10% KOH-88ml

Solution II

- CvEH (Crystal violet, Ethanol, H_2SO_4)
  Crystal violet-0.5gm
  70% Ethanol-80ml
  % H2SO4-20ml

Solution III

- GAW (Geimsa working solution +acetic acid+water)
  Geimsa working solution-2ml
  Acetic acid -10ml
  Deionised Water-88ml

Lactophenol cotton blue stain is compare with these three solutions for identification of fungi and observation of their colony morphology.

In our study we included Doctors who are associate professors, assistant professors, resident doctors and nurses.

All the participants involved in the study were given detailed information of the study and informed consent was taken in written format befor collecting swabs from their mobile screens and stethoscopes.

Inoculation of samples was done on SDA for fungal identification and colony morphology was observed by LPCB, MAK, GAW, CvEH mounts which were done. All tests were done thrice to rule out bias.

3. Results

The air samples in the hospital yielded different fungi like A*spergillus spp., Rhizopus, Yeast, Curvularia, Fusarium, Alternaria,* and *Penicillium* spp. Details are enlisted in tables 1 to 3.

Table 1: List of mycoflora

OPD	Fungus isolated from air	Fungus isolated from mobile	Fungus isolated from stethoscope	Fungus isolated from table
Pulmonary	Yeast	*Curvularia geniculata*	*Curvularia geniculata*	
Medicine	*Aspergillus flavus, Aspergillus fumigatus*	Nil	Nil	Nil
Surgery	Nil	Aspergilus flavus	Nil	Nil
Pediatrics	Nil	Aspergilus flavus	Aspergilus flavus	Rhizopus, Apophysomy
Radiotherapy	Aspergilus niger	*Rhizopus* spp.	Nil	Nil
General medicine	Nil	*Aspergillus niger*	*Aspergillus niger*	Nil
ENT	Nil	Nil	Nil	Nil
Physiotherapy	Nil	Nil	Nil	Nil
Ortho				
Neuro medicine	Rhizopus, Aspergillus Niger	Aspergilus Flavus	Acremonium	Nil
Neurology	Nil	Curvularia geniculata	Nil	Nil

Table 2: Recovery of fungi from air flora

Department	Fungus isolated from air	Fungus isolated from mobile	Fungus isolated from stethoscope	Fungus isolated from table
Microbiology	Yeast	*Aspergillus niger, Fusarium* spp., *Trichoderma*	Nil	Nil
Physiology	*Acremonium* spp., *Alternaria alternate*	Nil	Nil	Nil
Biochemistry	Nil	Fusarium	Nil	Nil

Table 3: List of fungal flora in wards.

Wards	Fungus isolated from air	Fungus isolated from mobile	Fungus isolated from stethoscope	Fungus isolated from table
General ward	*Aspergillus flavus, Fusarium* spp.	Aspergillus Niger	Aspergilus Flavus	Aspergillus Niger
Gynae ward	*Fusarium* spp.	*Aspergillus flavus*	Yeast	Nil
Trauma 1	Curvularia geniculata	Acremonium	Alternaria	Nil
Trauma 2	Alternaria	Nil	Alternaria	Nil
OT 1	*Penicillium* spp.,*Fusarium* spp, *Alternaria* spp.	*Alternaria* spp.	Nil	Nil
OT 2	*Aspergillus niger*	*Aspergillus niger*	Ni	Nil
PMR ward	Nil	Nil	Nil	Nil

In this study *Aspergillus* was found in high frequency. *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* were isolated from operation theatre and medicine on mycological media.

Penicillium was isolated as airborne fungus from operation theatre 1

Alternaria was isolated from stethoscopes of doctors.

Acremonium was isolated from mobiles of health care workers.

These Solutions had stability of maintaining morphology for two days.

4. Discussion

Lactophenol cotton blue has traditionally been used for study of fungal colony micromorphology. New solutions are required because of its hefty cost. The study of fungal load in

hospital environment is important because fugi are frequent causes of allergic and invasive nosocomial infections [8]. Hospital infections are commonly caused by fungi, like Candida sp and various species of Aspergillus, Cladosporium and Penicillium [8]. Such type of ocmprehensive has not been hitherto carried out in India. In a study from South India, it was found that the dominant fungal species identified were *Cladosporium* sp., *Aspergillus* sp., and *Alternaria alternata*, and the fungal flora had a seasonal pattern of occurrence [9]. There are some major diasadvantages of using Lactophenol cotton blue as mounting solution, one of which is its interference with adhesive material of sellotape while making sellotype mount from colonies [10]. As far as we now, this type of study has not been carried out in this part of the country, and new mounting fluids have not been tested for studying fungal colony. More such studies need to be carried out in order to effectively calculate the bioload of fungi in hospital environment and doctors' accessories.

5. Conclusion

Fungal load is very high in our hospital environment. Three new mounting fluids were as effective as LCB and can replace it in the long run.

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