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INVESTIGATION OF FUNGAL CONTAMINATION IN THE ENVIRONMENT OF INTENSIVE CARE UNITS OF A TERTIARY CARE HOSPITAL

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Abstract

Fungi are unicellular and multicellular eukaryotic organisms that can be found ubiquitously in soil, water and air. Their profound ability to live in multiple temperature ranges and places is attributed to their ability to produce pathogenic or non-pathogenic spores. Spores are resilient enough to withstand routine cleaning and disinfection practices. Presence of pathogenic spores in hospital environment, particularly in intensive care units, is critical in terms of immunocompromised patient safety who are being treated in such premises. Keeping in view the potential hazardous nature of fungi and their spores, the present study aimed to determine and identify different species of fungi responsible for air and surface contamination of the medical intensive care unit (MICU) and pediatric intensive care unit (PICU) environment and surfaces. For this, 70 samples from air and other surfaces (bed rails, instrument trays, floor and walls) were collected at MICU and PICU over a period of 1.5 months. Air samples were collected using the passive settle plate method, whereas samples from surfaces were collected using sterile swabs. Swabs were inoculated onto Sabouraud agar plates by the spread plate method and were incubated at 30-37 °C for 24 hours to 1 week, and were continuously observed to see the growth. Fungal growth on plates ensured fungal presence at that site. To identify the obtained fungal genera, growth cultures were examined for their morphological characteristics and then examined microscopically using the slide culture method. Slides were examined under the microscope for specific fungal microscopic structures. A total of 54 samples (77.14%) showed significant fungal growth, whereas 16 samples (22.85%) showed no growth. Among positive fungal samples, eight different types of fungi such as Neurospora, Candida spp, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Fusarium, Rhizopus and Mucor sp. were observed to be involved in contamination of surfaces and air of MICU & PICU. Relative to PICU, the MICU environment had increased fungal contamination as percentages of positive samples were 35.71% and 41.42% for PICU and MICU, respectively. The most abundant fungus recorded in both of the ICUs was Neurospora sp., followed by Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Candida spp., Fusarium, Rhizopus and Mucor species, with 30%, 24.28%, 11.42%, 10%, 7.14%, 7.15%, 5.71% and 2.85% relative percentages, respectively. Furthermore, the most contaminated site was observed to be the wall, followed by air, instrument tray, floor and bedrail, respectively. Presence of pathogenic fungal species in the environment of ICUs indicates the urgent need for stringent measures, particularly for the protection of resident immunocompromised patients of ICUs.

Keywords: *Fungal contamination, Medical intensive care unit (MICU), Neurospora and Aspergillus spp., Pediatric intensive care unit (PICU)*

INTRODUCTION

Fungi are a diverse group of eukaryotic single cellular or multicellular organisms forming a kingdom that is involved in disease manifestations, decomposition of pollutants and production of industrial products like medicines (1). Fungi are divided into two forms: yeasts and molds and further divided into five different phyla like Zygomycota (conjugation fungus), Deuteromycota (mitosporic and imperfect fungus), Ascomycota (Sac fungus), Mycophycophyta (lichen fungus) and Basidiomycota (club fungus) based on their different biological and morphological characteristic features (2).

There are certain fungi that are medically important as they can be pathogenic leading to cause different diseases in humans and animals. Among them the most prominent fungal species include *Candida* *albicans* and *Cryptococcus neoformans* that present as non-filamentous fungi & belong to phyla Ascomycota and Basidiomycota, respectively [2]. Among filamentous fungi *Aspergillus* spp., *Fusarium* spp., *Histoplsama* spp. *Penicillium* spp., Dermatophytes spp., Basidiomycetes spp., & Mucorales spp., are important that are responsible of different disease manifestations particularly in immunocompromised individuals (3, 4).

Pathogenic fungi in healthcare environment particularly in ICU air and surfaces pose great threat to the ICU patients due to their immunocompromised state. Prevalence of *Aspergillus* species, & *Candida* species in ICUs environments have been reported to cause severe outcomes in such patients (5, 6). Fungal species resistance to common antifungal drugs and transferring of their genotypes among environmental and clinical fungal isolates further worsen the situation for ICU patient survival. Other factors like prolonged hospital stay and undergoing intensive interventional procedures (transplants) aggravate probabilities of fungal progressions from environment to patient colonization/infection (5, 7).

There are certain factors which contribute in the presence and dispersal of fungal spores in the ICU environment. Among them dust, renovation activities and ventilation systems play significant role in fungal spread in the premises leading to enhance the risk of fungal infections among immune-compromised indwellers (8, 9). Furthermore, it has been noted that air, water systems and surfaces of hospital environment serve as reservoirs of fungal infections causing severe diseases like Aspergillosis and Candidiasis in ICU patients (5, 7, 8) highlighting the need of stringent antifungal disinfection practices.

There are certain practices and procedures that are being employed routinely to combat fungal microbial pathogens from the ICU environment but there is still need of more advanced and precise targeted approaches for fungal control. Proper monitoring, air cleaning systems using high efficiency particulate air (HEPA) filters (10), strict hygiene practices, use of personal protective equipment (PPE), separation of infected individuals, [8] applying antifungal stewardship program (5), close surveillance and fungal molecular epidemiological studies [9] play significant role in control of fungal diseases of ICUs and other health care locations.

Keeping in view the importance of fungal surveillance and evaluation of type of fungi involved in contamination of ICUs air and surfaces current study aimed to investigate the presence of different types of fungi in environment of intensive care units particularly Medical Intensive Care Unit (MICU) and Pediatric Intensive Care Unit (PICU) of a tertiary Care hospital of Rawalpindi. Main objective of the study was to determine & identify prevalent species of fungi involved in air and surfaces contamination of MICU and PICU premises.

METHODOLOGY

Study implemented prospective observational approach to collect data from air and surfaces of PICU and MICU during about three months. Convenience non-probability sampling technique was employed and 70 samples were collected for fungal isolation from five different sites of MICU and PICU premises. From each site of MICU & PICU 7 time sampling was performed during different time intervals to collect possible fungal species. Sample collection was performed in mornings of usual hospital days with routine flow of people and normal disinfection practices whereas sample collection was withheld on fumigation days.

MATERIAL PREPARATION

All the materials & equipment used to collect the samples were sterilized. Preparation of Sabouraud Dextrose Agar was done according to standard microbiological procedure and autoclaved at 121°C for 20 minutes. Another 150ml of autoclaved Sabouraud Dextrose Agar was taken in test tubes for slide culture method (11).

SAMPLE COLLECTION

Samples were collected from air, floors, bed rails, walls and instrument trays of MICU and PICU by using cotton swabs. Cotton swabs were soaked in sterile normal saline and then samples were collected by rubbing the swabs against the designated surfaces (from floors, bed rails, walls, instrument trays). Air samples were collected by passive settle plate method using 1/1/1/ scheme by placing exposed Sabouraud Dextrose agar plate with open lid at a distance of 1 m away from the wall, 1 m above the floor and for 1 hour to collect air sample. Passive settle plate method was used for detection of fungal contamination in the air (12) whereas cotton swabbing technique was used for surfaces contamination evaluation and detection (13) Cotton swabs were labelled during sample collection. Swabs were then sent to the laboratory in a container.

SAMPLE PROCESSING

Cotton swabs were inoculated on the culture plates by Spread Plate Method and labelled accordingly. Culture plates were kept under incubation at 25°C (room temperature) for one week and were monitored for fungal growth.

SLIDE CULTURE METHOD

"Slide culture method" was employed for identification of different fungi and it included slide preparation followed by microscopic examination. A drop of liquefied Sabouraud Dextrose agar was placed onto a clean glass slide. A small portion of fungal hyphae and spores from the fungal growth on agar plate was picked and stabbed into the solidified drop of Sabouraud Dextrose Agar on clean glass slide using a sterile inoculating loop. After inoculation into drop it was covered with a cover slip. Cover slip was pressed gently to avoid breaking the agar and to secure the drop. The slides were then placed in incubator at 30 °C for 3-5 days. Few cotton pieces soaked in normal saline were placed with slides to provide moisture for fungi to grow and to prevent agar drop from drying out. After incubation, the slides were examined under microscope to look for the type of hyphae, conidia, sporangia, conidiophores to identify fungal species [11].

RESULTS AND DISCUSSION

Total 70 samples were collected from MICU & PICU air & surfaces (35 samples from each of the ICU). Out of 70 samples from both of ICUs environment 54 samples were positive for fungal growth. Out of 35 samples collected from MICU, 29 samples were positive and 6 samples were negative for fungal growth whereas out of 35 samples collected from PICU, 25 samples were positive & 10 samples were negative for fungal growth indicating 41.42% & 35.71% prevalence of fungi in MICU & PICU premises, respectively (Table I). Current study findings comply with previous research showing similar results.

Table I. Number & percentages of positive/negative samples obtained from MICU & PICU environment

	MICU Positive samples	MICU Negative samples	PICU Positive samples	PICU Negative samples
Number of samples	29	6	25	10
Relative % of samples	41.42 %	8.57 %	35.71 %	14.28 %

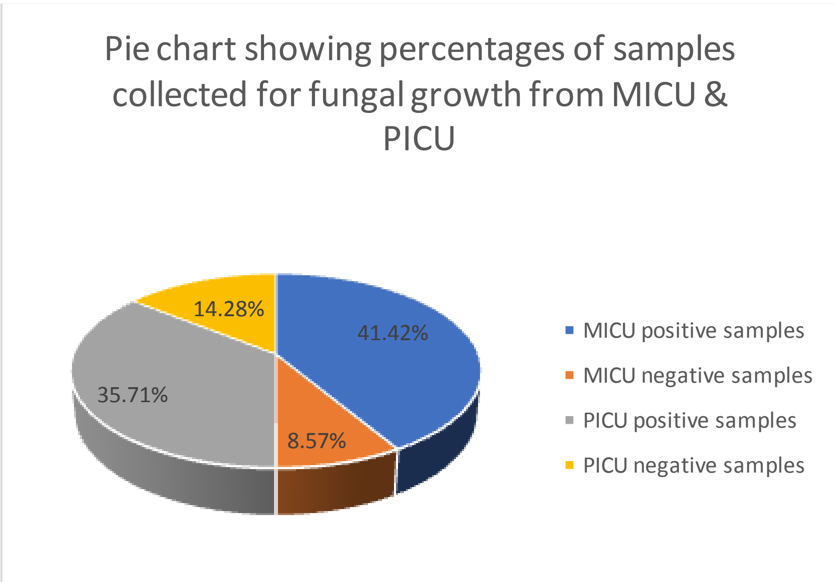

Fig. 1. Prevalence of fungi in MICU & PICU

From positive samples of MICU & PICU, eight different types of fungi were isolated & identified. Among isolated fungi *Aspergillus niger, Aspergillus fumigatus*, *Aspergillus flavus, Neurospora* spp., *Candida* spp., *Fusarium* spp., *Rhizopus* spp. and *Mucor* spp. were observed & recorded. Their genus-wise distribution in MICU & PICU samples was also recorded showing the most prevalent fungus was *Neurospora*, followed by *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*, *Fusarium* spp., *Candida* spp., *Rhizopus* spp. & *Mucor* species (Fig. 2). Isolation of different fungi from different surfaces of MICU & PICU have been illustrated (Table II).

Current study findings somehow comply with previous research showing similar results with highest prevalence of different *Aspergillus* species in ICU settings, (14) although in current study the top most prevalent genus was observed to be Neurospora spp. *Neurospora* presence is attributed to contamination of laboratories not the ICUs as it has not been mentioned in literature to cause fungal invasive diseases of humans (15). Furthermore, in pediatric ICU, presence of fungi like species of *Aspergillus*, *Fusarium* and *Candida* pose great threat to neonate and pediatric patients due to their weak immune response (16). Current study has observed similar results in PICU highlighting the need of much focused & precise adoption of antifungal disinfection strategies.

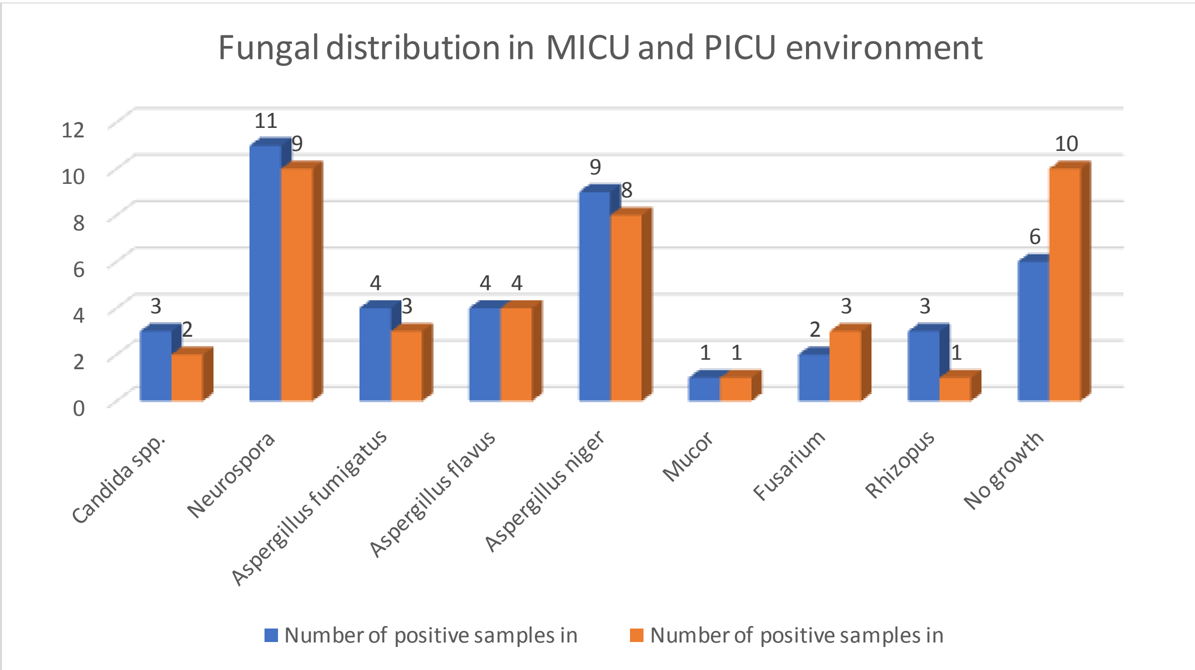

Fig. 2. Genus-wise fungal distribution in MICU & PICU environment

Table II. Number of positive samples & fungal genera obtained from air & different surfaces of MICU & PICU

ICU type	Area of sampling	Fungal genera isolated throughout sampling (7 samples each) Fungal isolates names	Positive samples	Negative samples
MICU	Walls	*Aspergillus fumigtus, A. niger, Mucor, Neurospora, Fusarium*	6	1
	Instrument Tray	*Aspergillus niger, A. flavus, Candida* sp., *Fusarium, Rhizopus*	6	1
	Bed Rail	*Aspergillus niger, A. flavus, Candida, Neurospora*	4	3
	Floor	*Aspergillus niger, A. flavus, A. fumigatus, Neurospora, Rhizopus*	6	1
	Air	*Neurospora, A. flavus, A. niger, A. fumigatus*	7	0
PICU	Walls	*Aspergillus flavus, A. niger, A. fumigatus, Neurospora, Candida*	7	0
	Instrument Tray	*Aspergillus flavus, A. niger, Neurospora, Candida*	5	2
	Bed Rail	*Fusarium, A. niger, A. fumigatus, Neurospora*	5	2
	Floor	*Neurospora, Fusarium, A. niger, A. fumigatus*	4	3
	Air	*Mucor, A. niger, Neurospora, A. flavus, Rhizopus*	4	3

As air & different surfaces of MICU & PICU were sampled for fungal contaminant evaluation, the walls were observed to be the most contaminated surfaces, followed by air, instrument trays, floor and bedrails when assessed collectively for both of MICUs & PICU surfaces (Table III). The high contamination of walls may be attributed to lack of routine daily cleaning of walls, while floors and other instruments are routinely cleaned and disinfected.

Table III. Distribution & prevalence of fungi isolated from different surfaces of MICU & PICU

Surfaces	Number of positive samples out of 7 in MICU	Number of positive samples out of 7 in PICU	Number of positive samples in MICU & PICU	Prevalence %	Negative samples of MICU and PICU
Walls	6	7	13	92.85	1
Floors	6	4	10	71.42	4
Bed rails	4	5	9	64.28	5
Instrument trays	6	5	11	78.57	3
Air	7	4	11	78.57	3

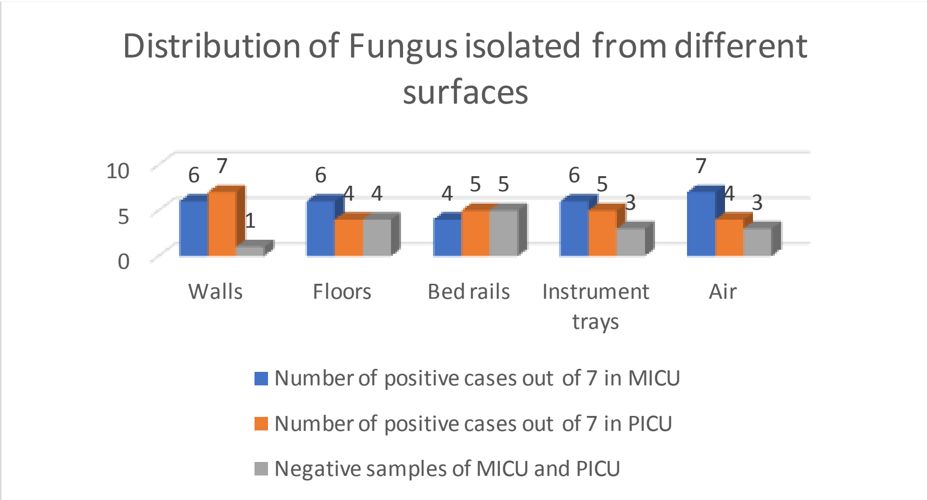

Fig. 3. Distribution of fungus isolated from different surfaces of MICU & PICU

When contamination of both of ICUs was compared then it was noted that fungal contamination was higher in MICU setting than in PICU. All the isolated fungal genera were observed to be much prevalent in MICU than PICU accept for the *Aspergillus niger* & genus *Fusarium* which were isolated in higher number from PICU than MICU environment (Fig. 4)

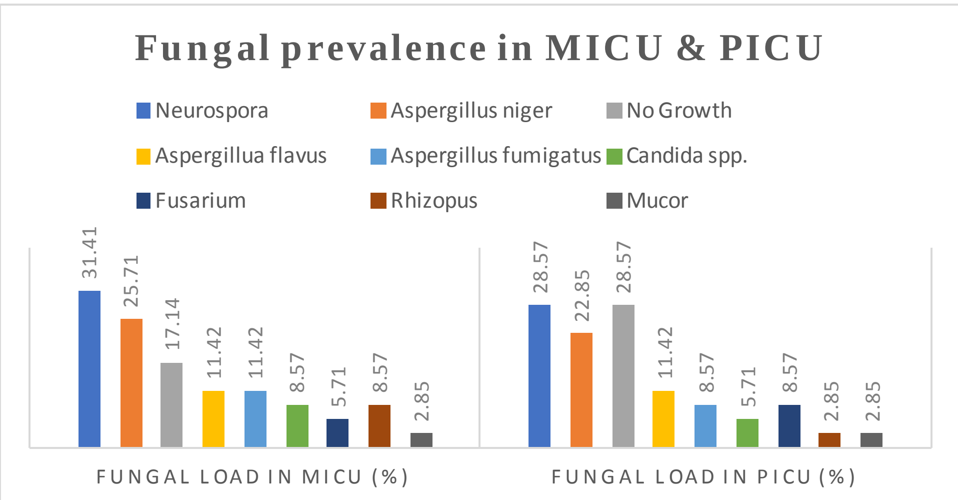

Fig. 4. Comparison of fungal load (prevalence) between MICU & PICU

When data from different surfaces was compared between two ICUs then it was noted that air was the most contaminated site & bedrails were the least contaminated sites in MICU whereas the walls of PICU were the most contaminated site and floor was the least contaminated site in PICU (Fig. 5). Previous research reported the floors to be the most contaminated sites (17). In current study floor contamination stood second after air contamination in MICU environment, and stood third in PICU settings whereas bedrails were stood the least contaminated & second most contaminated surfaces in MICU & PICU, respectively (Table III).

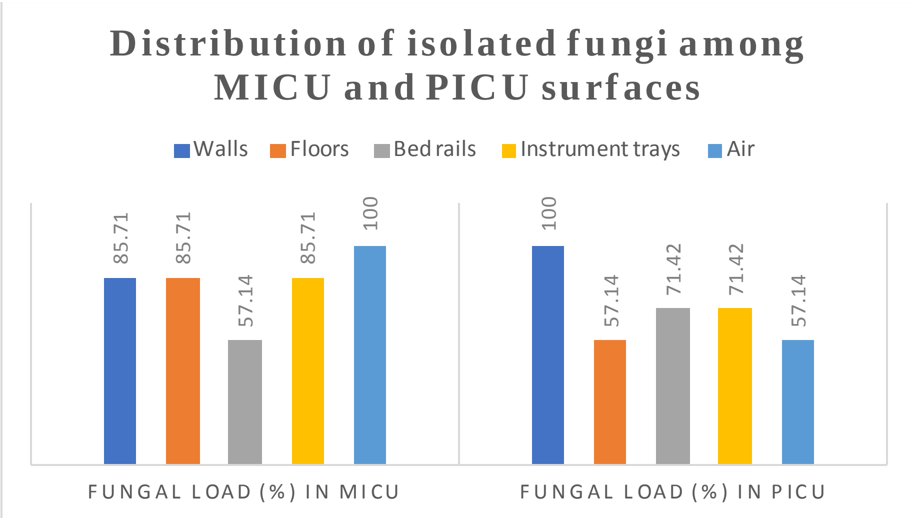

Fig. 5. Comparison of Fungal load (Prevalence) between different surfaces of MICU & PICU

Implications of presence of pathogenic and opportunistic fungi in healthcare settings specifically ICUs atmosphere are of great concern. As these fungi (*Candida* spp.,) & some emerging fungi (*Candida auris*) from atmosphere can easily colonize and infect immune-compromised patients of ICUs due to use of ventilator & prolonged hospital stay (18) *Candida* spp., like *C. auris* and *Aspergillus* spp., have been reported to complicate the antifungal treatments in ICU patients due to their antifungal drug resistance (19) So there is a need of proper regimen of antifungal disinfection strategies to avoid fungal invasiveness in ICU settings. For this established cleaning procedures must be intensively followed particularly for reusable medical equipment to avoid cross contaminations (10). Moreover, predictive weekly surveillance of fungi has been reported to be helpful in recognizing the fungal colonization patterns and subsequent fungemia development. So predictive surveillance approach can be used for timely management of any potential fungal pathogen (20).

CONCLUSION

As per the result of present study, it has been cleared that despite of routine disinfection practices in the ICU premises there is still a prevailing threat in the form of fungal spores or vegetative fungi. Presence of fungi poses serious risk to the indwelling immunocompromised patients in the ICUs. To overcome this scenario there is an urgent need to focus on disinfection practices particularly to combat fungal presence to avoid ICU patient complications and poor outcomes.

Authors' contribution:

KT conceptualize, manuscript write-up; NH, IR, NA research work; MU critical analysis.

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