Int.J.Curr.Microbiol.App.Sci (2016) *5*(7): 627-632

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 7 (2016) pp. 627-632**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.507.070

Emergence of Drug Resistant Fungi in Hospital Air: A Need for Strict Microbiological Surveillance

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Keywords

Drug resistant fungi, fungal nosocomial infections, fungaemia.

Article Info

Accepted:
20 June 2016
Available Online:
10 July 2016

A B S T R A C T

Fungal infections of hospital origin have been gaining importance in recent years due to their progressive increase with high rate of morbidity and mortality. Our study reports the results of environmental surveillance of fungi in specific areas of two tertiary care hospitals of Dr. S.N. Medical College, Jodhpur-Rajasthan. Study was conducted from January2015 to 2016. Samples from the air of two local tertiary care hospitals were collected on a monthly basis. The air sampling was done by passive sedimentation method on petriplates containing Sabouraud's dextrose agar medium supplemented with gentamycin for one hour in respective areas then sealed and sent to the Department of Microbiology for processing by standard microbiological procedures. This study find out different contamination levels at different areas of hospital with100 fungal isolates: *Candida albicans* (n =22), *C.parapsilosis* (n=15), *Candida tropicalis* (n=5), *Rhodotorula* (n =2), *Trichosporon* (n = 1), *Aspergillus* spp.(n =45), *Mucor* spp.(n=5) & *Penicillium* spp.(n=5). In general most of these isolates were found to be resistant to Flucanazole, Itraconazole & Clotrimazole while Voriconazole is still most sensitive. Opportunistic mycoses have been increased in recent years in our study as well as international health facilities. In our findings, *C.albicans, Candida parapsilosis*, non-Candida yeast species & many moulds were common which might be coming from human clinical material as well as from environmental samples. Results of our study were very similar to Raimunda Sâmia N. Brilhante *et al* study. Our study emphasize on strict hospital environmental monitoring for fungi to prevent possible fungal nosocomial infections because of our negligence about air contaminants fungi may causes severe opportunistic fungaemia with very high morbidity & mortality.

Introduction

The importance of bio-aerosols has been emphasized in recent decades due to their effect on human health (Gangneux *et al*., 2006). These particles act as transmitters of hospital infections (Dacarro *et al*., 2003; Afonso *et al*., 2006), as they act as epidemiologic markers of microbial contamination (Brasil *et al*., 2000). Fungal

infections of hospital origin have been gaining importance in recent years due to their progressive increase and their high rates of morbidity and mortality (Pfaller, 1996; Centeno *et al*., 2004). In the hospital environment, the airborne micro biota is formed mainly by filamentous fungi, especially the genera *Aspergillus*, *Penicillium* & *Mucor* (Rainer *et al*., 2001; Sanca *et al*., 2002) Yeasts of the genera *Candida, Rhodotorula,* and *Trichosporon* (Krajewska *et al*., 2004; Pini *et al*., 2005; Wang *et al*., 2005), all have been described as potential human pathogens & the important causative agent of hospital fungaemia (Moretti, 2007). The passive sedimentation technique is still widely recommended in the literature for use as a microbiological alert. Bio-aerosol monitoring in hospitals can provide information for epidemiological investigation of nosocomial infectious diseases and research in airborne microorganism spread and control, as well as for quality control measures (Moretti, 2007). This study involved the environmental monitoring of fungi in specific areas of two tertiary level hospitals.

Materials and Methods

The present study was conducted at Department of Microbiology, Dr. S.N. Medical College Jodhpur & attached group of Hospitals- Rajasthan. Air sampling from different areas of hospitals was performed at regular interval using the passive sedimentation method in 90 mm diameter 300 Petri dishes containing Sabouraud's dextrose agar medium supplemented with gentamycin. The plates were exposed in each of the environments for one hour positioned 2 meters high - roughly human respiration height (Pei-Chih *et al*., 2000). The Petri dishes were sealed and sent to the Department of Microbiology.

The samples were kept at 25 °C to 28 °C in B.O.D. incubator and examined after 48 hours to a week or so till any fungal - growth appeared. Different colonies of white, black, green and brown or cream colour were obtained. Yeasts were identified by germ tube test, characteristics morphology on Glucose agar-0.1%, culture characteristics on HI chrome agar (HI media, Mumbai, India) & confirmed by Automated Vitek-2 compact system.

Moulds were identified by texture &colour of the obverse & reverse surface of colony, Lactophenol cotton blue mount preparation & morphology found on slide culture. (Moline *et al*, 1999; Duguid *et al*, 1999; Forbes *et al.*, 2002; Freydiere *et al.*, 2001)

The antifungal susceptibility of the isolates was done by using CLSI M44 A-2 Disc diffusion method for Yeast on Mueller Hinton Agar with 2% glucose &0.5µg/ ml methylene blue & CLSI M38 A-2 broth micro-dilution method for Moulds.[16] (NCCLS 2004) Quality control was performed using standard strains of *Candida parapsilosis* ATCC 22019 for Yeasts & *Aspergillus flavus* ATCC204304 for Moulds.

Results and Discussion

A total of 300 air samples from 5 hospital environments were analyzed, from which 100 strains of fungi were isolated out of them *Candida albicans* (n =22), *C.parapsilosis* (n=15) *Candida tropicalis* (n=5) *Rhodotorula* (n =2), *Trichosporon* (n = 1) *Aspergillus* spp. (n =45) *Mucor* spp.(n=5) *Penicillium* spp.(n=5) were found as shown in Table-1 & Bar diagram.

Table.1 Frequency of fungi found in air of different areas of Hospital

Area of sampling	No. of petriplates	*Candida albicans* F%	*Candida parapsilosis F%*	*Candida tropicalis F%*	*Rhodotorula F%*	*Trichosporon capitatum F%*	*Aspergillus spp. F%*	*Penicillium spp. F%*	*Mucor spp. F%*
ICU of MGH	60	10	20	00	00	05	55	05	05
Burn unit of MGH	60	20	20	10	05	00	40	00	05
RRC OT of MGH	60	15	15	00	00	00	50	10	10
ICU of MDMH	60	15	20	15	05	00	40	00	05
Swine flu ICU of MDMH	60	50	00	00	00	00	40	10	00

MGH-Mahatma Gandhi Hospital, MDMH-Mathuradas Mathur Hospital Jodhpur

Table.2 Resistance pattern of Candida albicans isolates in %

Area of hospital	FLC	VRC	IT	AP	NS	CC	KTZ	MIC
ICU of MGH	75	00	75	25	35	50	50	50
Burn unit of MGH	50	00	50	25	40	80	50	40
RRC OT of MGH	75	10	50	20	25	60	25	50
ICU of MDMH	100	10	50	30	50	60	25	50
Swine flu ICU of MDMH	100	20	75	50	50	50	50	60

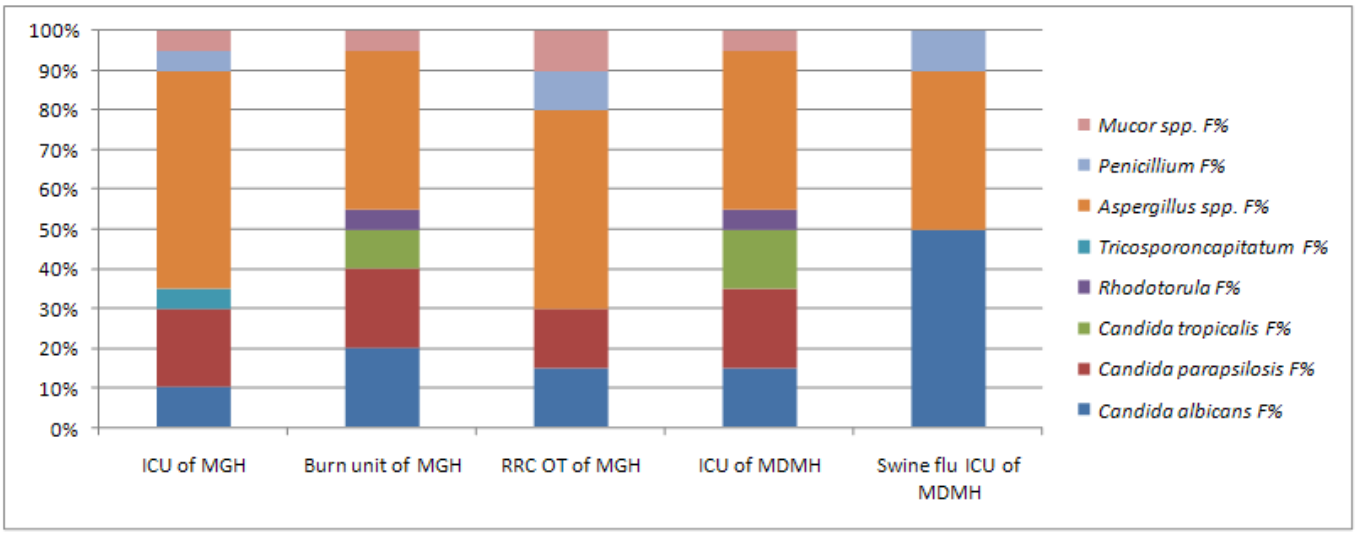
FLC-Flucanazole, VRC-Voriconazole, IT-Itraconazole, AP-AmphotericinB NS-Nystatin, CC-Clotrimazole, KTZ-Ketoconazole, MIC-Miconazole

Table.3 Resistance pattern of *Candida parapsilosis* isolates in %

Area of hospital	FLC	VRC	IT	AP	NS	CC	KTZ	MIC
ICU of MGH	50	00	50	25	25	50	50	40
Burn unit of MGH	100	10	75	35	50	80	50	50
RRC OT of MGH	50	10	50	10	25	55	25	50
ICU of MDMH	100	10	50	30	50	40	25	50
Swine flu ICU of MDMH	100	10	75	50	50	75	50	60

Table.4 Resistance pattern of Aspergillus spp. Isolates

Area of hospital	FLC	VRC	IT	AP	NS	CC	KTZ	MIC
ICU of MGH	80	00	50	25	25	75	20	30
Burn unit of MGH	100	20	75	50	50	100	50	40
RRC OT of MGH	75	10	50	20	25	60	25	40
ICU of MDMH	75	10	50	25	25	75	40	50
Swine flu ICU of MDMH	90	20	75	50	50	80	60	40



Bar diagram.1 showing Frequency of fungi found in air of different areas of Hospital

Regarding resistance pattern of isolates 80% *Candida albicans* and *C.parapsilosis* were resistant to Flucanazole, 60% to Itraconazole & Clotrimazole, 50% to Miconazole, 40% to Nystatin & Ketoconazole, while Voriconazole was found to be most sensitive in >90% followed by AmphotericinB in 70% isolates as shown in Table-2 and 3.

According to the literature, airborne particles can have many origins (Moscato, 2000). In environments with artificial ventilation, the air conditioning system, due to condensation trays, has been considered an important source of microorganism distribution. Thus, in environments with artificial ventilation, yeasts are probably transmitted by droplets produced by bio-aerosol equipment, as well as by coughing or sneezing, or even by routine procedures such as the withdrawal of vascular catheters. However, in environments with natural ventilation, the principal origins of air particles are believed to be ventilators,

nebulizers, air humidifiers, plant vases, some foods, and people themselves.

In conclusion, observations of our study strictly recommended that air monitoring is essential to prevent hospital infections. Among the preventive measures that should be considered are:- Good quality fumigation at regular interval, more frequent mechanical removal of dust, cleaning & mopping of floor & permanent items with disinfectant on daily basis, restriction of entry , better cleaning and maintenance of air conditioners and other equipment that produce bio-aerosols, correction of room humidity, use of protective clothing by hospital personnel, and better staff training regarding the threats of infection by airborne microorganisms.

Acknowledgements

We express grateful thanks to Principal & Controller & HODs of respective Departments of Mahatma Gandhi Hospital & Mathuradas Mathur Hospital of Dr. S.N. Medical College Jodhpur, Rajasthan.

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How to cite this article:

Seervi, K.L., P.K. Khatri, R.S. Parihar, Smita Kulshrestha, Saroj Meena and Archana Bora. 2016. Emergence of Drug Resistant Fungi in Hospital Air: A Need for Strict Microbiological Surveillance. *Int.J.Curr.Microbiol.App.Sci.* 5(7): 627-632.
doi: http://dx.doi.org/10.20546/ijcmas.2016.507.070