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Study on the Sources of Nosocomial Fungal Infections at Intensive Care Unit and Transplant Wards at a Teaching Hospital in Tehran

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Abstract

The incidence of nosocomial fungal infections has increased dramatically during the past two decades as the consequence of continuous increase in the number of severely immunocompromised patients. This study was done to determine the presumptive sources of nosocomial fungal infections at the intensive care unit and transplant wards (in a university- based teaching hospital in Tehran) during a 10-month period. Totally 583 samples were obtained from the air, surfaces, health care workers and also from the patients at those wards. Mycological culture of the samples yielded growth of 25 different genus and species of fungi and the most common isolated fungi were *Candida albicans*, *Penicillium* spp., *Aspergillus niger*, and *Cladosporium* spp., respectively. It was noted that health care workers were carrying fungi on their hands (50 %), nasal mucosa (57.6%), in oral cavity (38.6%) and also by their shoes (92.3%) and uniforms (92.7%). Environmental fungal contamination was shown and it was more prominent at the intensive care unit. Hospitalization also had more significant effect on colonization of fungi in the patients at the latter ward. Therefore, the highly susceptible patients in present study were at the greatest risk of developing fungal infections and preventive measures were critical for prevention and control of these life-threatening fatal infections.

Keywords: *Nosocomial, Fungal infection, Iran*

Introduction

Infections acquired during a hospital stay are called nosocomial infections. These infections can be bacterial, viral, and fungal or even parasitic (1). Advances in medicine by use of newer technologies and therapies have helped to treat patients suffering from previously devastating or fatal diseases but these successes have resulted in proliferation of a severely ill immunocompromised, hospitalized patient population. Furthermore, the AIDS epidemic has added to this growing population of immunocompromised individuals (2, 3). These immunocompromised patients are highly susceptible to nosocomial infections caused by organisms such as fungi that were previously considered to be of low virulence or non-pathogenic (3). Therefore, during the past two decades fungi have become increasingly important causes of nosocomial infections and have emerged as a frequent cause of mortality and morbidity in hospital patients (3- 5). Since nosocomial fungal infections (NFI) are often severe, rapidly progressive and difficult to diagnose or treat, there is a critical need for more efforts to be directed toward prevention, early diagnosis and aggressive treatment of these infections.

Among these, preventive measures are of major importance in the control of NFI. Therefore, full understanding of the epidemiology of these

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infections is crucial in the development of effective preventive strategies (6). In the present study a prospective surveillance was conducted over a 10-month period to determine environmental contamination by fungi and importance of carrying these organisms by health care workers (HCWs) at intensive care unit (ICU) and transplant wards. Indeed additional cultures were obtained from different specimens of patients for diagnosis of fungal colonization and infection in them.

Materials and Methods

This study was done over a 10-month period from June 2000 through April 2001 at the ICU and transplant wards at a university teaching hospital in Tehran, in order to show environmental contamination by fungi, carriage of these organisms by HCWs, fungal colonization and infection in the patients at those wards. Environmental contamination was examined by the air and surface sampling. In each ward, two surface samples were obtained (from the floors, walls, doors, windows, ventilation grills, washbasins, trolleys, bed uprights, blankets, pillows, bed sheets, and as well as the medical devices), before and after cleaning and disinfecting of the wards. Sampling was done by wiping premoistened cotton tipped sterile swabs over a surface measured approximately 20 cm^2, and then streaked on the Sabouraud's chloramphenicol agar (SC) and brain heart infusion agar (BHI) media. Sampling from the floors and walls was done by rubbing a piece of 4×4 cm carpet over those surfaces. In the next stage the carpet shaked down over the culture media. Clear sticky tape (sellotape) was used for the pillows, blankets and bed sheets sampling to remove adequate material for culture. It was done by applying 2×10 cm of the sellotape strips over those surfaces with gentle pressure for 10 seconds, then peeled it off and placed sticky side down onto the culture medium. SC and BHI media plates were used for air sampling. Two plates were used for a space of approximately 3×3×2 meter. We removed the lids of plates and put them about 8 min at the different parts of those wards. It is worthwhile to say that, we checked all the culture media for fungal contamination by putting them at 30^o C for 48h, before inoculation. To determine carriage of fungi by HCWs, hand wash cultures were performed by use of sterile polyethylene bags containing 25ml of BHI broth medium. The hands washed in the bags for about one min. Then the broth specimens were transferred into the sterile screw caped tubes and incubated at 30º C for 48 h. Subsequent subcultures were done onto the agar media. Nasal swabs were obtained and since blunt-scalpel is preferable so, this method (7) was used for oral sampling in HCWs. The shoes and uniforms of these people were sampled by use of swab and sellotape, respectively and all of the samples were cultured as previously mentioned. Fungal colonization and infection in the patients at those wards were studied by sampling from the oral cavity, nasal mucosa, ear, skin, vaginal discharge, urine, stool, blood and wounds during the admission and discharging time and also whenever clinical manifestation was present. All of the patients had been hospitalized for at least one week. Samples were obtained by swab and blunt scalpel and they were cultured as mentioned previously. Only blood samples were cultured by use of biphasic BHI medium. All the cultures on SC and BHI media, in this study were incubated at 25º C and 37º C, respectively and were daily checked for fungal growth. Isolated fungi were identified by their morphological and biochemical characteristics.

Results

During the period of 10-months, 583 samples were obtained from the environment, HCWs and patients at the ICU and transplant wards at a university teaching hospital in Tehran. Cultures of the samples yielded 1903 colonies of the 25 different genus and species of fungi. The list of isolated fungi is illustrated in Table 1. It

was noted that *Candida albicans* (20.9%), *Penicillium* spp. (19.1%), *Aspergillus niger* (16.2%) and *Cladosporium* spp. (12.9%) were the most common isolated fungi, respectively. To determine environmental fungal contamination, totally number of 128 air samples and 232 surface samples were obtained in this study. Sampling was done before and after cleaning and disinfecting of the wards to detect the effect of these procedures in decreasing the number of environmental fungal spores. Therefore, these samples were divided into two equal groups, 180 samples were obtained before cleaning and disinfecting of the wards and the rest obtained after these procedures. Table 2 shows that *Penicillium* spp. (27.5%), *Cladosporium* spp. (19.9%) and *Aspergillus niger* (16.3%) were the most common isolated fungi from the environment. By statistical analyzing (Mc Nemar-test) it was revealed that, cleaning and disinfecting procedures only had significant effect in decreasing fungal spores' population on the surfaces at the transplant wards and not in the other cases in this study. To present the role of HCWs in NFI, 130 samples were obtained from the 26 people at the ICU and transplant wards. Table 3 shows that *Aspergillus niger* (16.4%), *Penicillum* spp. (14%) and *Aspergillus* spp. (13.7%) were the most common recovered fungi. This study revealed that HCWs were carrying fungi on their hands (50%), nasal mucosa (57.6%), in oral cavity (38.6%) and by their shoes (92.3%) and uniforms (92.7%). A case of cutaneous pheohyphomycotic colonization by *Alternaria* sp. in a nurse at ICU was the other finding in this study.

In order to show the effect of hospitalization on fungal colonization, 14 patients were chosen from those wards and totally 92 samples were collected in two stages; 46 samples during admission and the rest at discharging time. Different genus and species of fungi were isolated (Table 4). *Candida albicans* (72.1%) and *Geotrichum candidum* (10.1%) were the most common isolated fungi, respectively. Statistical analysis by McNemar-test revealed that, only hospitalization had significant effect on fungal colonization in the ICU patients. Finally, *Rhizopus* sp. and *Candida tropicalis* were recovered from the amputation site in a patient with diabetic foot, hospitalized at ICU.

Table 1: Isolated fungi from the ICU and transplant wards

Organism	No. of Colonies	%
Candida albicans	397	20.9
Penicillium spp.	363	19.1
Aspergillus niger	308	16.2
Cladosporium spp.	245	12.9
Aspergillus spp.	108	5.7
Alternaria spp.	107	5.6
Rhizopus spp.	76	4
Geotrichum candidum	58	2.8
Mucor spp.	52	2.6
Rhodotorula rubra	52	2.6
Candida famata	34	1.8
Trichoderma spp.	33	1.7
Monilia sitophila	31	1.4
Trichosporon spp.	25	1.3
Cryptococcus albidus	15	0.8
Fusarium spp.	12	0.6
Candida tropicalis	10	0.5
Ulocladium spp.	9	0.5
Candida glabrata	7	0.4
Candida zeylanoides	7	0.4
Scopulariopsis spp.	2	0.1
Phoma spp.	2	0.1
Chetomium spp.	2	0.1
Arthrinium spp.	2	0.1
Candida crusei	1	0.05
Total	1903	100

Table 2: Fungi isolated from the environment of ICU and Transplant wards

Organism	Air		Surfaces		Total	
	No. of colonies	%	No. of colonies	%	No. of colonies	%
Penicillium spp	27	18.7	274	28.9	301	27.5
Cladosporium spp.	39	27	179	18.9	218	19.9
Aspergillus niger	5	3.4	173	18.2	178	16.3
Alternaira spp.	12	8.3	82	8.6	94	8.6
Rhizopus spp.	2	1.3	74	7.8	76	6.9
Aspergillus spp.	14	9.8	46	4.8	60	5.5
Mucor spp.	3	2.7	41	4.3	44	4
Monilia sitophila	16	11.1	15	1.6	31	2.8
Trichoderma spp.	2	1.3	22	2.3	24	2.2
Rhodotorula rubra	7	4.8	15	1.6	22	2
Fusarium spp.	0	0	10	1	10	0.9
Candida albicans	1	0.68	8	0.85	9	0.82
Geotrichum candidum	6	4.1	0	0	6	0.54
Ulocladium spp.	2	1.3	3	0.3	5	0.45
Trichosporon spp.	2	1.3	2	0.2	4	0.36
Cryptococcus albidus	4	2.7	0	0	4	0.36
Candida tropicalis	2	1.3	0	0	2	0.18
Scopulariopsis spp.	0	0	2	0.2	2	0.18
Chetomium spp.	0	0	2	0.2	2	0.18
Total	144	100	948	100	1092	100

Table 3: Fungi recovered from HCWs*

Organism	No. of colonies at				Total	
	ICU		Transplant ward			
	No.	%	No.	%	No.	%
Aspergillus niger	10	7.3	39	24	49	16.4
Penicillium spp	33	24.2	9	5.5	42	14
Aspergillus spp.	14	10.2	27	16.6	41	13.7
Rhodotorula rubra	16	11.7	14	8.6	30	10
Trichosporon spp.	0	0	21	12.9	21	7
Candida albicans	11	8	7	4.3	18	6
Cladosporium spp.	14	10.2	4	2.4	18	6
Candida famata	2	1.4	14	8.6	16	5.3
Alternaira spp.	6	4.4	7	4.3	13	4.3
Cryptococcus albidus	11	8	0	0	11	3.6
Trichoderma spp.	7	5.1	3	1.8	10	3.3
Rhizopus spp.	1	0.7	7	4.3	8	2.6
Candida zeylanoides	7	5.1	0	0	7	2.3
Mucor spp.	0	0	6	3.7	6	2
Candida glabrata	0	0	5	3	5	1.6
Fusarium spp.	2	1.4	0	0	2	0.6
Candida tropicalis	1	0.7	0	0	1	0.3
Candida crusei	1	0.7	0	0	1	0.3
Total	136	100	162	100	298	100

* HCWs: Health care workers

Table 4: Comparison of fungal colonization in patients during admission and discharging time

Organism	Admission time		Discharging time		Total	
	No.	%	No.	%	No.	%
Candida albicans	141	73	222	71.5	370	72.1
Geotrichum candidum	20	10.3	32	10	52	10.1
Penicillium spp	7	3.6	13	4	20	3.8
Aspergillus niger	2	1	16	5	18	3.5
Candida famata	3	1.5	15	4.6	18	3.5
Cladosporium spp.	4	2	5	1.5	9	1.7
Candida tropicalis	3	1.5	4	1.25	7	1.3
Aspergillus spp.	7	3.6	0	0	7	1.3
Ulocladium spp.	4	2	0	0	4	0.7
Candida glabrata	0	0	2	0.6	2	0.3
Phoma spp.	0	0	2	0.6	2	0.2
Rhizopus spp.	0	0	2	0.6	2	0.3
Arthrinium spp.	0	0	2	0.6	2	0.3
Total	193	100	320	100	513	100

Discussion

It is apparent that the incidence of NFI becoming more prominent by advances in medicine and growing population of immunocompromised or immunosuppressed patients.Fungal infections in these patients are often severe, rapidly progressive, difficult to diagnose or treat with high morbidity and mortality rate (2, 3). Therefore, preventive measures are of major importance in the control of these infections and require full understanding of the epidemiology of NFI (6). NFI may be acquired from the hospital environment (type I, hospital acquired) or developing during the course of hospitalization from a fungus previously colonizing or latently infecting a patient (Type II, hospital- associated) (5).

Hospital environment has been suggested to play a crucial role in the epidemiology of NFI. It is likely the sources of fungal spores at this area, are ventilation or air conditioning system, dust, decaying organic material, ornamental plants, flowers, fresh fruit, food, water and, particularly, building works in and around hospitals (6, 8). There are no limits to the number of fungal species in the environment, but filamentous fungi have been the main isolated fungal genera from the hospital environment (2). The spores of these fungi may spread through the air and remain airborne for prolonged periods. As a result, spores are ubiquitously found in the air and contaminate anything in contact with air. The process of settling out and becoming airborne again can repeat itself for prolonged period of time, because fungal spores will be viable for months in dry location. In presence of water spores will germinate and mycelial growth will occur with subsequent sporulation (6). Invasive filamentous fungal infections are a major infectious complication in patients with profound immunodeficiency. A majority of these infections is acquired by inhalation (9). It has been hypothesized that inhaled spores may become deposited in the upper and lower respiratory tract and led to subsequent pulmonary infection in immunocompromised patients (6, 8). Because these patients are extremely susceptible to local invasion of respiratory tissues by deposited spores, and there is subsequent dissemintation to other deep organs (3). Contamination of medical devices before or after shipment to hospital is the other mode of transmission (10). There is a strong positive correlation between nosocomial infections and

number, type and duration use of invasive devices and procedures (9). In the present study different genus and species of fungi were isolated from the air and surfaces and filamentous fungi were the most common isolated fungi (Table 2). Therefore, ICU and transplant wards patients in this study were at the greatest risk of developing NFI by exogenous sources as a result of inhalation fungal spores and a complex interaction between the patients underlying diseases and the use of invasive devices and procedures. NFI may be also acquired from the other sources. HCWs play an important role in the transmission of fungi to patients. *Candida* species are frequently isolated from the hands of these people and can be transmitted from hands to patients (11). Although 50% of HCWs harbor fungi on their hands but it was surprised that, *Candida.* spp. consisted 36.1% of these fungi and filamentous fungi were more prominent. Our results contrasted sharply with those of other studies (12). But cultures of the other samples that were obtained from the nasal mucosa, shoes and uniforms of HCWs in this study also yielded filamentous fungi predominantly. These finding could implicate to heavy contamination of environment by filamentous fungi and acquirement of these fungi by HCWs during working at those wards. Although isolation of fungi depends on a great number of factors (8) but other finding in our study like extremely contamination of uniforms and coat-hanger by *Penicillium* spp., conform to this hypothesis. It was shown that construction activity increases fungal spore concentration in the air and many investigators have described outbreaks of nosocomial invasive fungal infections, associated with construction or renovation activities in and around hospitals (6). During this study we noted that, windows of the wards were opened frequently and there was construction activity in the hospital next to the windows. Therefore, this activity could be the main source of environmental fungal contamination in our study.

As mentioned before, NFI may develop otherwise from a fungus previously colonizing the patient (Type II). *Candida* spp. are frequently encountered as a part of the human commensal flora and the majority of NFI (80%) are reported to be caused by these fungi (11, 3-18). It has been shown that *C.albicans* is the most frequently isolated *Candida.* spp. and mucosal surfaces are colonized with this fungus in up to 80% of hospitalized patients but tend to be significantly lower (2-37%) in healthy individuals. Since intensity of colonization with *Candidia.* spp. correlate with the subsequent severe *Canidia* infection, so hospitalization itself increases the risk of fungal infections (11). In present study *C. albicans* was the most common isolated fungi from the patients' samples and this proportion is comparable to that reported by the other studies (9, 19, 20). It is worthwhile to say that 35.7% of our patients were colonized with *C.albicans* in the meantime of hospitalization and hypercolonization with *Candida. spp*. also was seen during this time. HCWs probably played an important role in the colonizing of patients with *C.albicans*. Because they were carrying this fungus on their hands, nasal mucosa and in oral cavity. So, the patients in this study were at the risk of acquiring candidiasis from both endogenous (their own distinct strain of *Candida*) and exogenous (HCWs and environment) sources. It was shown that *C.tropicalis* and *Rhizopus* sp. were colonized on the wound of amputation site in a diabetic patient. Because this colonization may led to the serious infections (3, 11) this finding was a great risk factor in development of subsequent fatal infections in that patient. Although *C. tropicalis* and *Rhizopus* sp. also were isolated from the HCWs' and environmental samples, but further studies (by molecular typing techniques) were needed to determine the main sources of the isolated fungi from the patient in our study.

Overall this study showed that highly susceptible patients were at the greatest risk of developing fungal infections. Therefore, continued epidemiological and laboratory research is needed to better characterize fungal infections

and allowing to improve preventive, diagnostic and therapeutic strategies for NFI. Our results underline the importance of environmental surveillance and strict application of cleaning procedures and measures that can prevent fungal contamination of the wards. It was noted that these measures should be applied to the whole of the ward and not only to patient's room. The results presented here clearly show that training of the HCWs and improving their knowledge about fungal infections also has special importance in prevention and controlling of NFI.

Finally, according to the results, during the past four years environmental controls by equipping necessary facilities accomplished beyond the personal training of those wards. Therefore further similar investigations would be able to show the effectiveness of application of those measures for prevention and NFI control in those wards in future.

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