

Seasonal Distribution of Fungi in Soil Found in Two Hospitals in Bandar Abbas, IranElham Shahbazy¹, Negin Azizi¹, Parivash Davoodian², Khojasteh Sharifi_Sarasiabi³, Afsaneh Karmostaji⁴

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Type of article: Original**Abstract**

Introduction: Fungal contamination in hospitals has been a major health concern, especially in immunosuppressed patients. Construction programs increase the risk of fungal contaminations in hospitals. This study aimed to monitor and compare fungal contamination in two hospitals in Bandar Abbas, Iran, one of which was undergoing construction. Furthermore, the study determined the seasonal variations in the prevalence of fungi identified in the two hospitals.

Methods: This study was conducted during April-December 2014 on 146 soil samples collected from the Shahid Mohammadi Hospital that was under construction and the Shariati Hospital that was not involved in any construction program. The soil samples were collected in the morning from the dust on the ground. Statistical analysis was performed using the chi-squared test.

Results: Among the fungal cultures, 83.56% were found to be positive. We identified 122 fungal isolates, representing 16 genera, 13 of the genera were isolated from Shahid Mohammadi Hospital and 14 were isolated from Shariati Hospital. *Aspergillus* was the dominant fungus in both hospitals. In Shariati Hospital, 100% of *Aspergillus niger* were isolated in the summer. In the spring, Mucor was dominant in both hospitals, and, in the winter, *Rhizopus spp.* was the dominant fungus in Shahid Mohammadi Hospital (55.5%); however, this fungus was dominant in spring in Shariati Hospital (66.7%). In Shariati Hospital, *Scopulariopsis*, *Drechslera*, *Penicillium spp.*, and Yeasts were present only in the fall, whereas yeast was the dominant fungus in summer (100%) in Shahid Mohammadi Hospital. There were not significant differences between two hospitals, with the exception of *Fusarium spp.*, which was more frequent in Shariati Hospital, where no renovation was taking place ($p = 0.008$).

Conclusion: Our results indicated that specific ecological niches existed in the two general hospitals in Bandar Abbas. Special attention should be paid to environmental control programs. It is recommended that soil exposure be considered in patients' histories in addition to the current focus on infections related to food and water.

Keywords: fungi, soil microbiology, hospital, seasons

1. Introduction

It seems that we are faced with the threat of increased incidence of invasive fungal infections. A study conducted in 2007 involving 1265 Intensive Care Units in 75 countries around the world showed that 19% of pathogens in patients admitted to the Intensive Care Unit (ICU) were fungi (1). The prevalence of fungal infections in ICUs in

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Iran in 2011 was 1.9% with a mortality rate of 58.8% (2). Since construction at the site of the hospital was causing fungal spores to be dispersed throughout the environment, the overall construction-related mortality was reported to be as high as 50% in 2015 (3). Another study conducted in 2015 indicated that invasive fungal infections were responsible for the deaths of 1.4 million people annually (4). Immunosuppressed patients, such as patients with HIV infections are at high risk of fungal contamination, because fungal spores can be inhaled and cause local or invasive infections. The main fungal genera responsible for these infections are *Aspergillus spp.*, *Fusarium spp.*, *Scedosporium spp.*, and *Mucorales* (5). *Penicillium marneffei* was the agent for fatal systemic mycosis in patients infected with HIV in Southeast Asia, especially during the rainy season (6). Furthermore, a variety of non-dimorphic, soil-related yeasts and molds, including *Rhizopus* and *Mucor*, can cause significant local or systemic infections in people. The sources of fungal spores are soil, decaying vegetation, and dust (7). In hospital environments, soil and dust are transmitted through ventilators and air conditioning systems, and decaying materials, food, and water are possible sources of fungal spores that can cause nosocomial infections. Particularly, construction work in and around hospitals increases the risk of nosocomial infections caused by airborne fungi (5). With respect to the seasonal variations of fungi, some European studies have reported seasonal distributions of *Cladosporium spp.*, *Alternaria spp.*, and *Aspergillus spp.* in outdoor air (8). Invasive fungal infections, depending on their types and the risk factors of patients, can result in a fivefold increase in death, cause extended hospitalization up to 19.2 days, and increase costs to between 55,400\$ (9) to € 9,36- €13,24\$ (10). At the time of this study, Shahid Mohammadi Hospital in Bandar Abbas, Iran, was involved in a large renovation program that included the construction of buildings. Our aim was to monitor fungal contamination in the Hospital during the construction period and compare it with another hospital that was not involved in any construction program during the same period. Furthermore, we evaluated the seasonal variations of the main fungi identified in these hospitals.

2. Material and Methods

2.1. Research design and setting

This study was conducted during April-December 2014 using hospital soil samples that were collected from two hospitals in Bandar Abbas, Iran. Bandar Abbas is a port city, is the capital of Hormozgan Province, and has approximately 500,000 inhabitants (11). The average temperature range in winter is 17 - 25 °C, and it is 35 - 40 °C in the summer, with a relative humidity of about 85%. Shahid Mohammadi Hospital is a 400-bed University Hospital in Bandar Abbas, and, at the time of this study, the Hospital was involved in a large renovation program, including the construction in hospital. The other hospital in Bandar Abbas that was involved in this study was the Shariati Hospital, and it had no construction taking place at the time of this study. We collected 146 soil samples from 54 locations at the two Hospitals, with 53 samples from the site of the Shariati Hospital and 93 samples from the site of the Shahid Mohammadi Hospital. The samples were taken randomly at 12-m intervals at each site (12). The soil samples were taken from a depth of 5 to 8 cm at each location.

2.2. Data collection and methods

Soil samples consisting of 3-5 grams, each were collected in the morning from the dust that had settled down during the night. Twenty-five samples were taken in the winter at temperatures of 17-25 °C, and 23 samples were collected in the summer at temperatures of 32-37 °C. Twenty samples were collected in the spring, and 25 samples were collected in the fall. Three-to-five grams of each soil sample were taken from the air conditioning ducts, corridors, and garden soil at a depth of 5-8 cm. The samples were removed using a sterile wooden spatula, and they were placed into sterile petri dishes. Each soil sample was given a code that contained related information, such as temperature, moisture content, pH, type of vegetation, redox potential, location in the shade or sun exposure and transferred to the Mycology Laboratory of the School of Medicine at Hormozgan University of Medical Sciences. The soil samples were dried in air at 25 °C for seven days. We added 3-to-5 grams of the soil samples to test tubes that contained 25 ml of sterile physiologic saline (0.9% NaCl) at 25 °C, stirred the mixture for 5 min, and waited 25 min for the soil to settle. In order to inhibit the growth of bacteria, 8 ml of the supernatant were added to another sterile test tube that contained 2 ml of streptomycin and penicillin antibiotic solutions (2 mg/L), mixed the contents, and incubated them for 30 min. This solution was mixed again, and 0.5 ml of it was transferred to a Sabouraud's dextrose agar medium (BioMerièux, Marcy-1, Etoil, France) that contained 0.5 g/L of cycloheximide, and we incubated at the mixture at 25 °C for two weeks (13). Statistical analysis was performed using the chi-squared test.

3. Results

Among the 146 soil samples in the study, 83.56% of them were positive for fungal cultures. Altogether, 122 fungi isolates were diagnosed, representing 16 genera. Thirteen genera were isolated from the samples taken at Shahid

Mohammadi Hospital and 14 genera were isolated from samples taken at Shariati Hospital. Eleven genera were found at both of the hospitals (Table 1).

Table 1. Frequency (%) of fungi isolated from the two hospitals

Isolate	HA; n ¹ (%)	HB; n ² (%)	Total; n ³ (%)	p-value
Aspergillus spp.	10 (24.39)	20 (24.47)	30 (24.59)	0.98
Aspergillus niger	3 (7.32)	15 (18.52)	18 (14.75)	0.069
Mucor spp.	5 (12.19)	9 (11.11)	14 (11.47)	0.81
Rhizopus spp	3 (7.32)	9 (11.11)	12 (9.83)	0.35
Penicillium spp	2 (4.88)	8 (9.87)	10 (8.19)	0.17
Alternaria spp.	3 (7.32)	4 (4.94)	7 (5.74)	0.48
Epicoccum	4 (9.76)	3 (3.70)	7 (5.74)	0.08
Fusarium spp.	5 (12.19)	2 (2.47)	7 (5.74)	0.008
Yeasts	1 (2.44)	3 (3.70)	4 (3.28)	0.60
Mycelia Sterilia	0	3 (3.70)	3 (2.46)	0.05
Drechslera spp	1 (2.44)	2 (2.47)	3 (2.46)	0.98
Psylomyces	1 (2.44)	2 (2.47)	3 (2.46)	0.98
Absidia	0	1 (1.23)	1 (0.82)	0.26
Scopulariopsis	1 (2.44)	0	1 (0.82)	0.11
Phoma	1 (2.44)	0	1 (0.82)	0.11
Ascozarpe	1 (2.44)	0	1 (0.82)	0.11

1: From total of 81; 2: From total of 41, 3: from total of 122; HA: Shariati Hospital; HB: Shahid Mohammadi Hospital

Table 2. Incidence and seasonal variation of different types of fungi isolated from the soil of Shariati Hospital and Shahid Mohammadi Hospital in Bandar Abbas

Fungal Species Isolated	Winter		Spring		Summer		Fall		Total	
	HA n (%)	HB n (%)	HA n (%)	HB n (%)	HA n (%)	HB n (%)	HA n (%)	HB n (%)	HA n (%)	HB n (%)
Aspergillus sp	2 (20)	6 (30)	5 (50)	6 (30)	1 (10)	2 (10)	2 (20)	6 (30)	10 (24)	20 (25)
Aspergillus niger	0	4 (27)	0	3 (20)	3 (100)	4 (27)	0	4 (27)	3 (7)	15 (18)
Mucor spp.	1 (20)	2 (22)	2 (40)	5 (55)	1 (20)	2 (22)	1 (20)	0	5 (12)	9 (11)
Rhizopus spp	1 (33)	5 (55)	2 (67)	2 (22)	0	1 (11)	0	1 (11)	3 (7)	9 (11)
Penicillium spp.	0	2 (25)	0	1 (12)	0	3 (37)	2 (100)	2 (25)	2 (5)	8 (10)
Alternaria spp.	1 (33)	0	1 (33)	1 (25)	0	0	1 (33)	3 (75)	3 (7)	4 (5)
Epicoccum	4 (100)	1 (33)	0	1 (33)	0	0	0	1 (33)	4 (10)	3 (4)
Fusarium spp	3 (60)	1 (50)	1 (20)	0	0	0	1 (20)	1 (50)	5 (12)	2 (2)
Yeasts	0	0	0	0	0	3 (100)	1 (100)	0	1 (2)	3 (4)
Scopulariopsis	0	0	0	0	0	0	1 (100)	0	1 (2)	0
Drechslera spp.	0	1 (50)	0	0	0	0	1 (100)	1 (50)	1 (2)	2 (2)
Psylomyces	1 (100)	0	0	1 (50)	0	0	0	1 (50)	1 (2)	2 (2)
Phoma	0	0	0	0	0	0	1 (100)	0	1 (2)	0
Ascozarpe	1 (100)	0	0	0	0	0	0	0	1 (2)	0
Mycelia Sterilia	0	1 (33)	0	0	0	1 (33)	0	1 (33)	0	3 (4)
Absidia	0	0	0	0	0	0	0	1 (100)	0	1 (1)

HA: Shariati Hospital; HB: Shahid Mohammadi Hospital

The genus *Aspergillus* was the most frequent, occurring in 30 samples (24.59%), and it was followed by *Aspergillus niger* (14.75%), *Mucor spp.* (11.47%), and *Rhizopus spp.* (9.83%). Except for *Fusarium spp.* ($p = 0.008$), there were no significant differences between the frequencies of fungi isolated in the samples from the two hospitals ($p > 0.05$). The incidence and seasonal variations of the fungi isolated from the soil of the two hospitals are as follows. The highest rate of fungal isolation (30.33%) in both hospitals was in the winter, it was greater at Shariati Hospital than at Shahid Mohammadi Hospital (34.14% versus 28.40%). The minimum isolation occurred in the summer (17.2%), with Shariati Hospital (12.20%) being less than Shahid Mohammadi Hospital (19.75%). Tables 2 indicates the seasonal variations of the different types of fungi isolated in the two hospitals. *Aspergillus* was the dominant fungus in both hospitals during the year. At Shariati Hospital, 100% of the *Aspergillus niger* species were isolated in the summer. In the spring, *Mucor* was dominant in both hospitals, and, in the winter, *Rhizopus spp.* was the dominant fungus at Shahid Mohammadi Hospital (55.5%). This fungus was dominant in the spring at Shariati Hospital (66.7%). At Shariati Hospital, *Scopulariopsis*, *Drechslera*, *Penicillium spp.*, and *Yeasts* were only observed in the fall. However, yeast

4. Discussion

In this study, we described the seasonal variations of the fungi that were isolated from a large number of soil samples collected from Shahid Mohammadi Hospital and Shariati Hospital in Bandar Abbas, Iran. We compared the fungal distribution in the two hospitals while one of them was undergoing construction activities and the other was not. However, contrary to our expectations, there were no significant differences between the fungi populations at the two hospitals with the exception of *Fusarium spp.*, which occurred in larger concentrations at the hospital where there was no construction activities taking place. Although the main objective of this study was investigate the presence of pathogenic fungi in the soils of the hospitals, the most common fungi were saprophytic soil-related fungi. Many studies in Iran have shown the presence of fungi in the air (14, 15). Although, many reports from around the world have shown that soils are important sources of fungi in hospitals (5, 8, 13, 16-18), there is little information about fungal flora in the soil in Iran. In the soil of Qazvin, the most fungi were isolated from the genus *Cladosporium spp.* (29.69%) with *Aspergillus spp.* at 22.52%. The high incidence of *Cladosporium* isolates was in contrast to our findings in which no *Cladosporium spp.* was isolated. However, the prevalence of *Aspergillus spp.* identified in our study was close to the value reported for the soil in Qazvin.

Penicillium spp. has been reported as the third most predominant soil-borne fungus (with a prevalence of 19.90%) in Qazvin (13), and it was 52% of the fungi isolated from soil samples from hospitals in Sari, Iran (15). Our results were in contrast with the reported values in that we found that *Penicillium spp.* accounted for 8.19% and was ranked fifth among the fungi recovered from soil samples. Another study in Sari in 2001 showed that the most common keratinophilic fungi isolated from forest soil were *Chrysosporium* and *Microsporium gypseum* and the most geophilic dermatophytes were *Trichophyton terrestand* *Trichophyton aioli* (19). Among the potential pathogens that were identified, *Mucor spp.* and *Rhizopus* were the two most rapid growing fungi, so that the frequencies of *Mucor spp.* and *Rhizopus* in soil of two hospitals were respectively 11.47% and 9.83%, and these fungi can cause infections in immunosuppressed, malnourished, or severely-burned people (13). We did not find the *Chrysosporium* species as was the case in other studies in Iran conducted by Hedayati et al., Moallaei et al., Shadzi et al. and Mahmoudabadi (14, 15, 20, 21). These studies reported that the incidence of this keratinophilic fungus was 3.7%, 8.40%, 41.6% and 54.2%, respectively. The explanation for this may be the different climate of Bandar Abbas. Although the presence of allergenic and pathogenic fungi in the soil does not necessarily mean that they are causing problems for patients, but their presence does indicate that they present potential risk factors for particularly sensitive patients. In addition, the results of our study led to a better understanding of the pattern of soil-borne fungi, and this information may be helpful for physicians, allergists, and epidemiologists. A study of the seasonal distribution of fungi within a hospital located in Bandar Abbas showed that the outdoor fungal load was far higher in the winter. This result was inconsistent with the result of a French study that indicated that the fungal load was higher in the fall (22). Bandar Abbas is hot and humid, and the maximum temperature in the summer can reach 49 °C (120 °F); the minimum temperature in the winter usually is in the range to 8 to 10 °C (41-43 °F) with a mean relative humidity of 65%. These climatic characteristics provide a suitable condition for fungal growth in the winter. This study is unique because there was no information on the background levels of fungal concentrations in outside soil samples during the period of construction at Shahid Mohammadi Hospital. Culture-based methods have some limitations in detecting some fungal genera, and Polymerase Chain Reaction is recommended to overcome these limitations.

5. Conclusions

The results of this study indicated that there is a higher fungal load in winter and that there were no significant differences between the two hospitals with respect to the presence of fungi, with the exception of *Fusarium spp.* that was more abundant at Shariati Hospital where no construction was taking place. Our results indicated the presence of specific ecological niches in the two general hospitals in Bandar Abbas. That was due to ability that fungi have to grow in conditions of environmental stress, such as direct sunlight, high temperatures, low levels of moisture, and a range of pH values in the soil, which could allow them to be associated with soil-related, opportunistic infections. Since, a variety of soil-related bacterial and fungal pathogens can cause serious diseases in people, it is recommended that soil exposure be routinely considered in patient histories, along with food- and water-born infections, when infectious diseases are included in the diagnosis.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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