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Full Length Research Paper

The seasonal distribution of airborne fungi in two hospitals in Istanbul

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Changes in fungal concentrations depend on seasonal and environmental conditions. The aim of this study was to determine the seasonal distributions of the fungal flora in Dr. Siyami Ersek Chest, Heart and Vascular Center Education and Research Hospital (SEH) and Kartal Yavuz Selim State Hospital (KYSH) in İstanbul. Samples were collected seasonally from different stations (microbiology laboratory, toilet, patient waiting saloons, hospital garden and library) in these hospitals. Distributional patterns of the isolated micro fungi were evaluated for each season. In SEH, the highest number of the fungi was isolated in summer, followed by autumn, spring and winter, respectively. Among the 257 micro fungi isolated from this hospital, the most common species were *Alternaria alternata* (25.8%), *Cladosporium cladosporioides* (21.9%), *Penicillium glabrum* (12.9%), *Penicillium brevicompactum* (7.4%) and *Cladosporium herbarum* (3.9%). In KYSH, the highest number of fungi was isolated in summer followed by autumn, spring and winter, respectively. Among the 221 micro fungi isolated from this hospital, the most common species were *Cladosporium cladosporioides* (19.5%), *Alternaria alternata* (15.8%), *Cladosporium herbarum* (11.3%) and *Penicillium brevicompactum* (9.0%).

Key words: Istanbul, airborne fungi, hospital flora, seasonal flora.

INTRODUCTION

Organic and inorganic particles are ubiquitous in atmosphere one of which is fungi. These important constituent of atmosphere can cause health problems in human, animal and plants (Kalliokoski, 2003; Afzal et al., 2004; Çolakoğlu, 2004; Awad, 2005) owing to being easily diffused by air (Kalliokoski, 2003; Ilhan and Asan, 2001). Fungi are found not only in air but also in soil (Ilhan and Asan, 2001) and water (Asan et al., 2003). Seasonal conditions affect the spore counts of fungi in the atmosphere (Simsekli et al., 1998; Medrela-Kuder, 2003). As moisture and temperature differ according to the seasons, types and densities of the fungi in the air vary from seasons to season (Liao et al., 2004) and one geographical feature to another (Abdel et al., 2007). They play significant roles in the decomposition of decaying organic matter in nature, in enzymes, organic acids, antibiotics, proteins, as well as, vitamin production; however they may cause many diseases (Menezes et al., 2004; Asan et al., 2010).

Aspergillosis is one of the most seen diseases (Fleischer et al., 2006). Fungi also lead to some disorders, such as allergic illnesses, asthma and sinusitis (Ronan et al., 2005; Topbas et al., 2006). Also, crowded places for instance, hospitals are suitable environments for fungal infection (Li and Hou, 2003). Therefore, such places should be monitored in routine intervals (Sarica et al., 2002). Fungal infections have higher hazardous health risk particularly for immunocompromised patients such as cancer patients and human immune virus (HIV) carriers (Weems et al., 1987; Bouakline et al., 2000). Some types of fungi also cause nosocomial infections mostly resulting in death (Pei-Chih et al., 2000; Yücel and Kantarcioglu, 2001). It is important to know the fungal densities in hospitals and immunocompromised patients' homes. The first study performed on hospital air flora was carried out in Edirne Trakya University Hospital (Sarica et al., 2002); however such studies are not rather limited in our country. Therefore, we aimed in this study to determine the airborne fungal flora of two hospitals in İstanbul and we intended to show allergenic fungi here.

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MATERIALS AND METHODS

This study was carried out in Dr. Siyami Ersek Chest, Heart and Vascular Center Education and Research Hospital (SEH) and Kartal Yavuz Selim State Hospital (KYSH) in İstanbul. Samples were taken monthly from five different stations (microbiology laboratory, toilet, patient waiting saloons, hospital garden and library) in the hospitals for every month during year 2005 to 2006 using Petri-dish method by opening Petri plates for 15 to 30 min at a height of 75 to 85 cm. Rose Bengal-Pepton Dextrose with streptomycin was used for isolation. Rose Bengal-Pepton Dextrose Agar contains: dextrose, 2 g; pepton, 5 g; KH_2PO_4; $MgSO_4.7H_2O$, 0.5 g; agar, 15 g; distilled water, 1000 ml. After this, the compound was sterilized at the temperature of 120°C for 15 min, 30 mg/L Rose bengal was added so that the diameter of the fungus colonies did not expand and then 30 mg/L streptomisin was added to prevent the bacteria reproduction (Sarica et al., 2002). The Petri plates used to collect samples were incubated for 7 days at room temperature (22 to 26°C). Thereafter, the grown colonies were subculture to potato dextrose agar (PDA), Sabouraud dextrose agar (SDA) and Czapek's agar (CZA) media. During the incubation, colony morphologies (color of surface and reverse, exudation, etc.) were noted.

Fungal predations were obtained using Lactophenol solution and Lactophenol- cotton blue solution (Bilgehan, 2002) for microscopic micro fungal identifications using a binocular light microscope. Each structure of micro fungi was measured at least 50 times and then their averages were calculated. Domestic and foreign textbooks and literatures were used for identification of genera and species. The resource named 'illustrated genera of imperfect fungi' was used in distinguishing the genus (Barnett and Hunter, 1987). '*Mucorales*' for the identification of *Rhizopus* species (Zycha and Siepmann, 1969), 'The genus *Aspergillus*' for the identification of *Aspergillus* species (Raper and Fennel, 1965), 'A manual of the Penicillia' for the identification of *Penicillium*, *Gliocladium*, *Paecilomyces* species (Raper et al., 1949), 'A revision of the genus *Trichoderma*' for the identification of *Trichoderma* species (Rifai, 1969), 'Dematiaceous Hypomycetes' for the identification of *Alternaria*, *Cladosporium*, *Ulocladium*, *Aureobasidium*, *Scopulariopsis* species (Ellis, 1971), 'The genus *Fusarium*'' for the identifications of *Fusarium* species (Booth, 1971), 'The genera of fungi sporulation in pure culture' for the identification of *Acremonium* species (Arx, 1981), 'Ainsworth and Bisby's Dictionary of the fungi' and 'Tohumsuz Bitkiler Sistematiği'; (Bacteriophyta, Cyanophyta, Phycophyta, Mycophyta, Lichenes) for the identification of *Mycelia sterilia* (Ainsworth et al., 1973; Çolakoğlu, 1999) were used.

İstanbul is a city generally affected by the Mediterranean climatic conditions (http://istanbul.meteor.gov.tr/). According to the observations recorded by Kandilli Observatory in İstanbul, the annual average temperature is 13.7°C. The average temperature is -5°C in January and 22.7°C in July. The annual precipitation is 789 mm and 38% of this is in winter, 18% in spring, 13% in summer, 31% in autumn but different results were obtained from Göztepe, Sariyer, Kartal, Şile, Florya, Yeniköy and Kumköy weather observation stations. The Anatolian part of İstanbul is a bit warmer than the Rumelian part (http://www.koeri.boun.edu.tr/). The temperature and moisture values measured in the studied hospitals during the study are given in Table 1.

RESULTS

In SEH, 24 micro fungus species belonging to 14 genera were identified and 257 micro fungus colonies were isolated in total (Tables 2 and 3). The lowest number of the isolated fungi was found in winter. The most common isolated fungal genus was *Alternaria* (27%) and the fewest isolated fungus genera were *Acremonium, Fusarium, Scopulariopsis* and *Trichoderma* (0.4%). The most common isolated fungus species was *Alternaria alternata* (25.8%) (Table 3). The most common micro fungi in terms of their seasonal distributions were *Alternaria, Cladosporium, Penicillium* and *Aspergillus* (Figure 1). In KYSH, 24 species belonging to 9 genera were identified and 221 microfungi colonies were isolated in total (Tables 4 and 5). The highest number of the fungi was isolated in summer (30.0%), followed by autumn (28.5%) and winter (17.6%), respectively. The fewest number of isolated fungi was in winter (17.6%). In KYSH, the most isolated fungus was *Cladosporium* (32.6%) while the rarest isolated fungus was *Gliocladium* (0.5%). The most isolated fungus species was *Cladosporium cladosporioides* (19.5%) (Figure 2).

DISCUSSION

The number of fungal spores in air changes since the seasonal conditions have quite effects on the fungus concentration. In one study in Taiwan, the fungus concentration in air in summer was found to be 20 times higher than in winter (Çolakoğlu, 1983). The highest numbers of fungi were isolated in summer in both hospitals, followed by autumn, spring and winter, respectively (Figure 3). When the air temperature and moisture in İstanbul is considered, summer is the most suitable season for fungal growth compared to spring, autumn and winter (Çolakoğlu, 2004). In many other studies performed in our country, the same seasonal ranking appeared, summer yielding the highest numbers of fungal isolations (Simsekli, 1997; Cetinkaya et al., 2005; Topbas et al., 2006). The optimum temperature and humidity for a fungal growth were 20 to 24°C and 50% humidity. Nonetheless, they still reproduced well above 65% moisture value (Çolakoğlu, 2004).

In our study, the optimum environmental conditions for the isolation of the microfungi were obtained in summer, autumn and spring (Table 1). In a similar study in USA, it was found that the largest number of fungus isolation was in summer and spring and the lowest number of isolation was in winter and spring (Shelton et al., 2002). In one study in Pakistan, it was reported that the seasonal climate conditions, in particular moisture and temperature, directly affected the fungus density (Medrela-Kuder, 2003). Our study indicates that the most isolated species in SEH were *Alternaria alternata* (25.8%), *Cladosporium cladosporioides* (21.9%), *Penicillium glabrum* (12.9%), *Penicillium brevicompactum* (7.4%), *Cladosporium herbarum* (3.9%) and *Aspergillus niger* (3.5%). In KYSH, the most frequent isolated species were *C. cladosporioides* (19.5%), *Alternaria alternate* (15.8%), *Cladosporium herbarum* (11.3%),

Table 1. Humidity and temperature values measured in Dr. Siyami Ersek Chest, Heart and Vascular Center Education and Researh Hospital and Kartal Yavuz Selim State Hospital during the study period.

Month	1				2				3				4				5			
	A		B		A		B		A		B		A		B		A		B	
	T	H	T	H	T	H	T	H	T	H	T	H	T	H	T	H	T	H	T	H
February 2005	18	48	17	50	16	47	16	54	15	48	14	53	8	50	8	53	15	48	16	55
March 2005	19	54	17	55	19	55	17	53	16	54	18	52	10	55	9	56	18	54	17	56
April 2005	19	41	18	42	18	41	17	42	17	40	16	43	12	40	11	41	19	40	15	42
May 2005	21	77	19	80	20	78	19	79	19	78	18	79	21	79	20	80	21	79	17	80
June 2005	24	70	22	70	22	69	23	69	23	69	22	66	21	70	21	69	23	69	21	68
July 2005	26	66	25	75	26	66	25	75	27	67	25	74	27	70	25	75	26	66	25	75
August 2005	24	65	25	65	26	65	27	66	25	67	26	67	26	70	26	66	27	65	27	67
September 2005	23	75	24	72	25	74	23	73	24	73	22	74	23	77	22	76	25	76	25	76
October 2005	18	83	16	96	18	83	14	94	19	83	14	95	15	82	11	88	18	83	13	94
November 2005	23	94	16	94	20	94	15	93	19	94	16	94	14	95	14	52	22	93	19	92
December 2005	18	57	15	59	19	58	14	55	17	58	14	58	8	60	8	60	16	59	17	59
January 2006	16	40	15	42	15	40	15	43	17	40	16	41	5	41	5	40	17	41	16	41

A: Dr. Siyami Ersek Chest, Heart and Vascular Center Education and Researh Hospital; B, Kartal Yavuz Selim State Hospital; T, temperature; H, humidity; 1, microbiology laboratory; 2, toilets; 3, patient waiting saloons; 4, hospital gardens; 5, library.

Penicillium brevicompactum (9.0%) and *Penicillium commune* (5.9%). Among these, *A. alternata* and C. *cladosporioides* were found in both hospitals during all sampling seasons. The occurrence of these species in indoor air environments was previously reported in another study in Turkey (Aydogdu et al., 2005). The numbers of the isolated microfungi (*Cladosporium, Alternaria*, *Aspergillus* and *Penicillium*) were found to be high in some countries (Lugauskas and Krikstaponis, 1999; Rainer et al., 2001; Ahdikari et al., 2004).

The most common isolated micro fungus in SEH was *Alternaria* (27.0%) followed by *Cladosporium* (26.2%), *Penicillium* (25.0%) and *Aspergillus* (9.0%). These microfungi comprise 87.2% of all the micro fungi isolated. In KYSH, the most common genera were *Cladosporium* (32.6%), *Penicillium* (23.1%), *Alternaria* (19.9%) and *Aspergillus* (10.0%), comprising 85.6% of all the fungi isolated in this hospital. In many studies, *Cladosporium, Alternaria, Penicillium* and *Aspergillus* (Pei-Chih et al., 2001; Sarica et al., 2002) were isolated.

While the largest number of *Alternaria* was isolated in summer, it was isolated rarest in spring and winter. It was isolated from both hospitals. This situation was determined in a study in Greece (Pyrri and Kapsanaki, 2007). *Cladosporium* was isolated mostly in summer and less in winter (Aydogdu and Asan, 2008). *Penicillum* was observed in high numbers in winter in İstanbul (Çolakoğlu, 1996). Moreover, *Aspergillus* was also high in concentration in autumn and less in winter (Gelincik et al., 2005). 257 micro fungi colonies were isolated from SEH whereas 221 microfungi colonies were obtained from KYSH. Unlike KYSH, SEH contained *Acremonium* (0.4%), *Fusarium* (0.4%), *Paecilomyces* (0.4%), *Trichoderma* (0.4%) and *Ulocladium* (1.6%).

Fungi lead to disorders like allergic illnesses, asthma and sinusitis in humans (Gelincik et al., 2005). The allergen fungi are *Cladosporium* (Zureik et al., 2002), *Alternaria*, *Aspergillus* and *Penicillium* (Çolakoğlu, 2004; Menezes et al., 2004)). In our study, these fungi were isolated at high level. For immunosuppressive patients, fungus infections are very important and can result in death. Therefore, exposure to fungal contaminants can be prevented using high-efficiency particulate arresting (HEPA) filtration systems in hospitals (Rainer et al., 2001). Fungal concentrations were found to be proportional to

Table 2. The seasonal distribution of the microfungal genera in SEH during the study.

Genus name	Spring	Summer	Autumn	Winter	Total	Percentage (%)
Acremonium	0	0	0	1	1	0.4
Alternaria	14	31	19	5	69	27.0
Aspergillus	7	4	9	3	23	9.0
Aureobasidium	2	5	0	0	7	2.7
Cladosporium	19	30	13	5	67	26.2
Fusarium	0	0	0	1	1	0.4
Gliocladium	0	0	3	0	3	1.2
Mycelia sterilia	0	4	5	0	9	3.5
Paecilomyces	0	0	1	0	1	0.4
Penicillium	19	3	16	26	64	25.0
Rhizopus	1	1	2	2	6	2.3
Scopulariopsis	1	0	0	0	1	0.4
Trichoderma	1	0	0	0	1	0.4
Ulocladium	0	3	1	0	4	1.6
Total	64	81	69	43	257	100
Percentage (%)	24.9	31.5	26.8	16.7		

According to the data obtained, the highest number of the fungi was isolated in summer (31.5%) followed by autumn (26.8%) and winter (16.7%), respectively.

Table 3. Colony number and percentages of total microfungus species in Dr. Siyami Ersek Chest, Heart and Vascular Center Education and Research Hospital during the study.

Genus and species name	Number of colonies	Percentage (%)	Month of isolation	Place of isolation
Acremonium	1	0.4	2	c
Acremonium sp	1	0.4	2	c
Alternaria	69	27.0	1,2,5,6,7,8,9,11,12	a, b, c, d, e
Alternaria alternata	66	25.8	1,2,5,6,7,8,9,11,12	a, b, c, d, e
Alternaria tenuissima	2	0.8	7	c
Alternaria citri	1	0.4	2	d
Aspergillus	23	9.0	1,2,3,4,7,8,11,12	a, b, c, d, e
Aspergillus candidus	3	1.2	1, 12	d
Aspergillus flavus	2	0.8	7, 8	c
Aspergillus nidulans	5	2.0	3, 4	a, b, d
Aspergillus niger	9	3.5	1,2,7,11,12	c, d, e
Aspergillus versicolor	4	1.6	3	b, e
Aureobasidium	7	2.7	3, 4	c
Aureobasidium pullulans	7	2.7	3, 4	c
Cladosporium	67	26.2	1,3,4,5,6,7,8,9,10,11,12	a, b, c, d, e
Cladosporium cladosporioides	56	21.9	1,3,4,5,6,7,8,9,11,12	a, b, c, d, e
Cladosporium herbarum	10	3.9	1,6,10,12	c, d
Cladosporium sphaerospermum	1	0.4	6	d
Fusarium	1	0.4	2	d
Fusarium nivale	1	0.4	2	d
Gliocladium	3	1.2	10	a, c
Gliocladium roseum	3	1.2	10	a, c
Mycelia sterilia	9	3.5	6,8,9,10	d
Paecilomyces	1	0.4	10	e
Paecilomyces variotii	1	0.4	10	e
Penicillium	64	25.0	1,2,3,5,6,7,10,11,12	a, b, c, d, e
Penicillium brevicompactum	19	7.4	1,11,12	b, d

Table 3. Contd.

Penicillium citrinum	6	2.3	2,5,11	a, c, d
Penicillium commune	4	1.6	3,10	a, b, e
Penicillium digitatum	2	0.8	2	e
Penicillium glabrum	33	12.9	5,6,7,10	c, d
Rhizopus	6	2.3	1,3,12	d
Rhizopus nigricans	6	2.3	1,3,12	d
Scopulariopsis	1	0.4	3	d
Scopulariopsis brevicaulis	1	0.4	3	d
Trichoderma	1	0.4	4	d
Trichoderma viride	1	0.4	4	d
Ulocladium	4	1.6	8, 9	d
Ulocladium botrytis	4	1.6	8, 9	d
Total	257	100		

1,January; 2, February; 3, March; 4, April; 5, May; 6, June; 7, July; 8, August; 9, September; 10, October; 11, November; 12, December; a, microbiology laboratory; b, toilets; c, patient waiting saloons; d,hospital gardens; e, library.

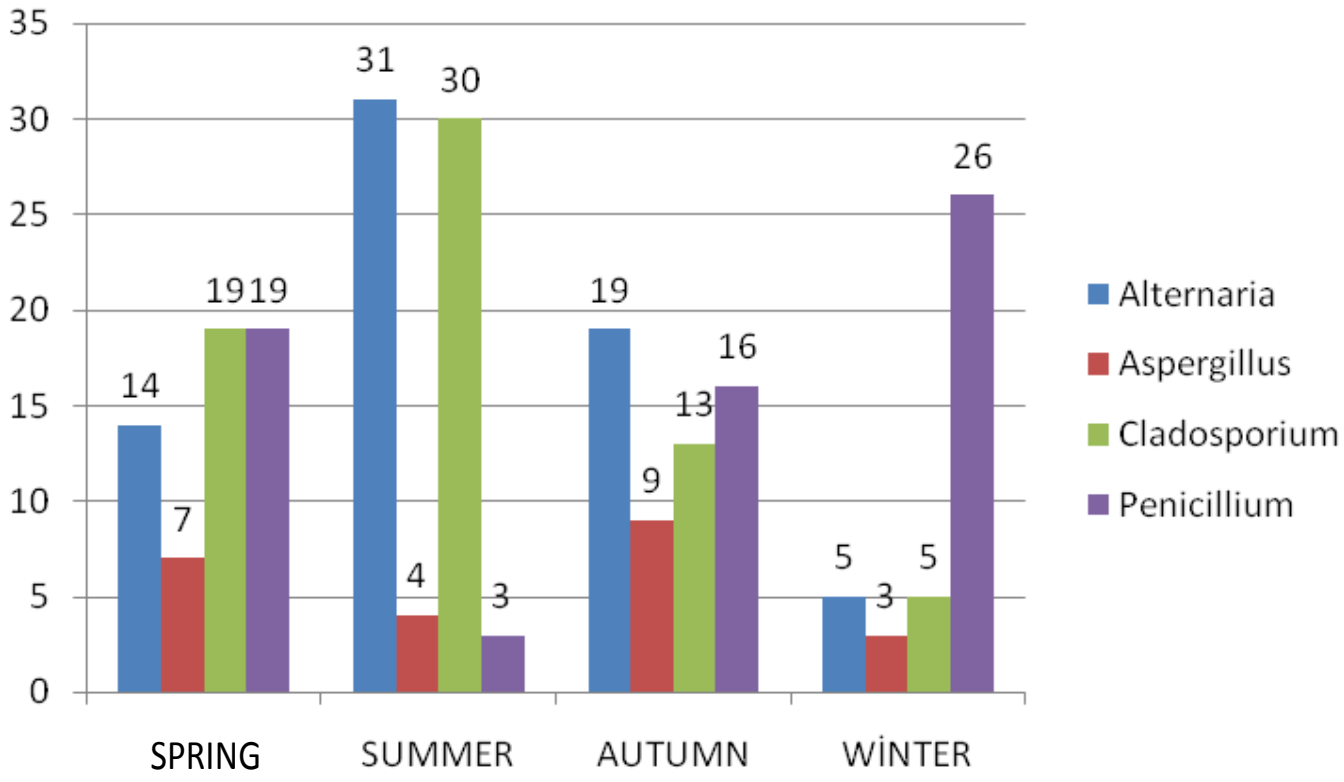

Figure 1. The seasonal distribution of the most common microfungal genera in SEH.

the temperature and moisture.

It increased or decreased depending on the seasonal conditions. *Cladosporium, Alternaria, Penicillium* and *Aspergillus* were dominantly isolated. As a result, the airborne fungal flora should regularly be determined in hospitals to prevent fungal infections.

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Table 4. The colony number and percentage rates of the microfungal genera isolated in KYSH during the study.

Genus name	Spring	Summer	Autumn	Winter	Total	Percentage (%)
Alternaria	8	21	11	4	44	19.9
Aspergillus	7	2	11	2	22	10.0
Aureobasidium	1	8	2	1	12	5.4
Cladosporium	21	29	13	9	72	32.6
Gliocladium	1	0	0	0	1	0.5
Mycelia sterilia	4	0	4	0	8	3.6
Penicillium	7	3	22	19	51	23.1
Rhizopus	1	2	0	3	6	2.7
Scopulariopsis	3	1	0	1	5	2.3
Total	53	66	63	39	221	100
Percentage (%)	24.0	30.0	28.5	17.6		

Table 5. Colony numbers and percentages of microfungal species in Kartal Yavuz Selim State Hospital during the study.

Genus and species name	Number of colonies	Percentage (%)	Month of isolation	Place of isolation
Alternaria	44	19.9	1,5,6,7,8,9,12	a,b,c,d,e
Alternaria alternata	35	15.8	1,5,6,7,8,9,12	a,b,c,d,e
Alternaria tenuissima	9	4.1	5,6,9	c,d
Aspergillus	22	10.0	2,3,6,9	a,c,d,e
Aspergillus candidus	3	1.4	2	e
Aspergillus cervinus	1	0.5	2	e
Aspergillus flavus	2	0.9	2	d
Aspergillus fumigatus	1	0.5	2	a
Aspergillus nidulans	3	1.4	3	d
Aspergillus niger	8	3.6	2,3,6,9	c,d,e
Aspergillus niveus	1	0.5	3	e
Aspergillis reptans	1	0.5	2	c
Aspergillis restrictus	1	0.5	3	d
Aspergillus versicolor	1	0.5	3	d
Aureobasidium	12	5.4	1,3,2,6,7,10,12	c,d
Aureobasidium pullulans	12	5.4	1,3,2,6,7,10,12	c,d
Cladosporium	72	32.6	1,2,3,5,6,8,9,10,11,12	a,b,c,d,e
Cladosporium cladosporioides	43	19.5	1,2,5,6,8,9,12	a,b,c,d,e
Cladosporium herbarum	25	11.3	1,2,3,5,9,10,11,12	a,c,d,e
Cladosporium sphaerospermum	4	1.8	5	d
Gliocladium	1	0.5	4	d
Gliocladium roseum	1	0.5	4	d
Mycelia sterilia	8	3.6	5,10,11	d
Penicillium	51	23.1	1,2,3,5,8,10,11,12	b,c,d,e,
Penicillium brevicompactum	20	9.0	2,10,11	c,d,e,
Penicillium citrinum	11	5.0	2,3,5,10,11	b,c,e
Penicillium commune	13	5.9	3,8,10,11	c,d,e
Penicillium glabrum	7	3.2	1,10,11,12	b,d
Rhizopus	6	2.7	1,2,3,6,12	d
Rhizopus nigricans	6	2.7	1,2,3,6,12	d
Scopulariopsis	5	2.3	2,3,4,5	c,d

Table 5. Contd.

Scopulariopsis brevicaulis	2	0.9	4,5	d
Scopulariopsis brumptii	3	1.4	2,3,4	c
Total	221	100		

1,January; 2, February; 3, March; 4, April; 5, May; 6, June; 7, July; 8, August; 9, September; 10, October; 11, November; 12, December; a, microbiology laboratory; b, toilets; c, patient waiting saloons; d, hospital gardens; e, library.

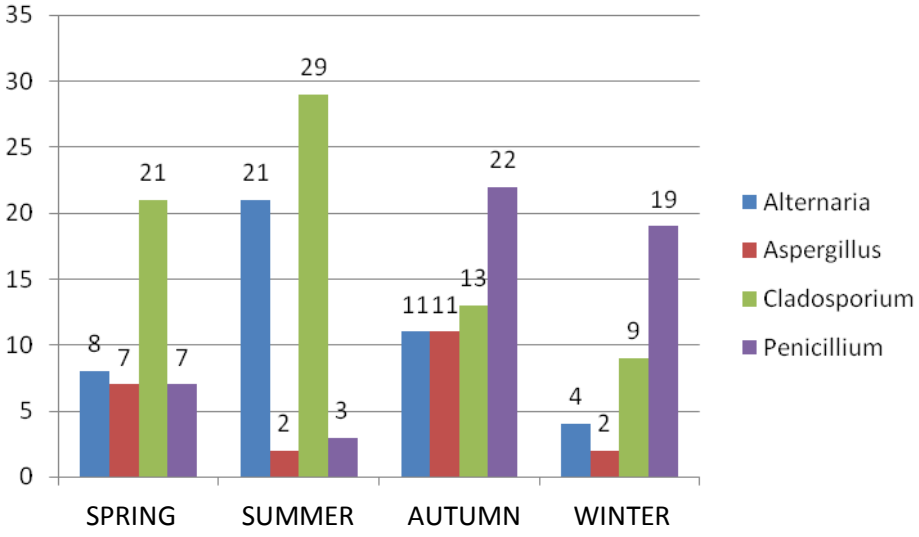

Figure 2. The seasonal distribution of the most common microfungal genera in KYSH.

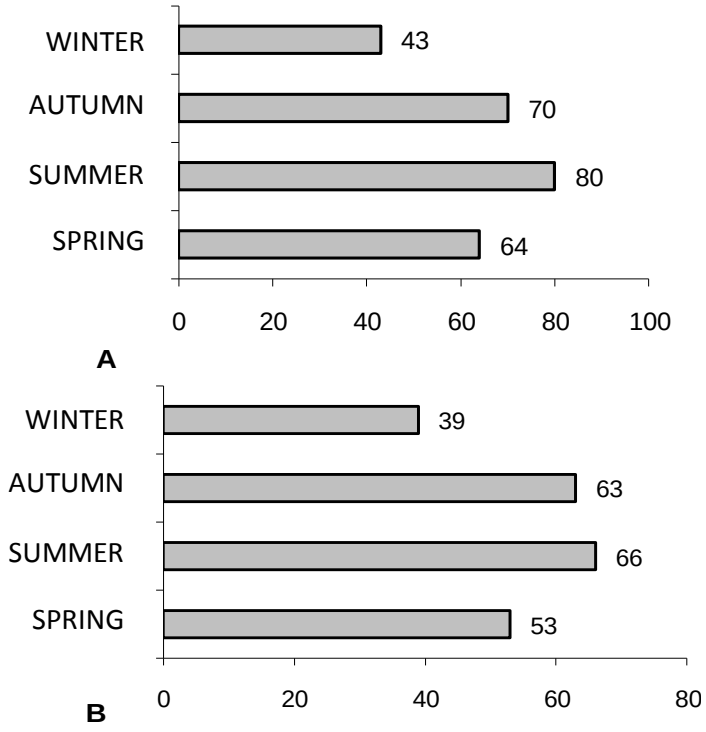

Figure 3. Seasonal distribution of the isolated fungi in SEH and KYSH. (A) SHE, (B) KYSH.

the study.

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