

Airborne fungi and bacteria in indoor and outdoor environment of the Pediatric Unit of Edirne Government Hospital

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Abstract This study was performed between January 2004 and December 2004 in 13 stations in the Pediatric Unit of Edirne Government Hospital in order to determine the outdoor and indoor airborne microfungal and bacterial contents. The results of air samplings revealed that 1,376 micro-fungal and 2,429 bacterial colonies in total were isolated. The isolated microfungal specimens were identified and 65 species from 16 genera were determined. Among these, the most frequent genus was *Cladosporium* with 462 colonies (33.58%) followed by *Alternaria* with 310 (22.53%) and *Penicillium* with 280 (20.35%) colonies. The isolated bacterial samples were grouped based on their Gram-staining properties. The most frequent ones were Gram (+) cocci with 1,527 colonies (62.87%) followed by Gram (+) bacilli with 828 colonies (34.09%) and Gram (−) bacilli with 74 colonies (3.05%). *Staphylococcus*, *Bacillus*, *Corynebacterium*, and *Micrococcus* appeared to be the common genera isolated for all months. Statistical analyses were performed in order to see if there existed a relationship between meteorological conditions and the microfungal and bacterial species and their concentrations.

Keywords Airborne fungi · Airborne bacteria · Hospital indoor air · Hospital hygiene · Allergen · Biomass · Pediatrics

Introduction

Airborne microflora in hospital rooms was the subject of numerous studies as a potential case of hospital infections (Ferrin et al. 2001; Letrilliart et al. 2001; Augustowska and Dutkiewicz 2006). Air-control measures are crucial for reducing dissemination of airborne biological particles in hospitals. Monitoring is strongly recommended for epidemiological investigation in epidemic situations, as nosocomial outbreaks have been linked to airborne transmission of pathogens. Such monitoring can be used to detect increases in fungal spore density during periods of high risk; for example, during building construction work (Gangneux et al. 2006).

Indoor air of a hospital may affect not only the patients' healths but also that of the hospital staff in relation with patients. Monitoring of indoor air quality of health foundations is a first step condition in prevention of nosocomial infections. Today, such a monitoring is performed according to the presence of surface microorganisms, but air sampling for microbial and particulate counts is also suggested (Klanova and Hollerova 2003).

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While hospital staff, patients, and their visitors are the main sources of airborne microorganisms in a hospital environment, some as fungal spores originate from outdoors. The more the number of occupants in buildings, the more the microbial load in these buildings. Therefore, microbial load is rather variable and depends on the number of occupants and the type of the works done inside (Beggs 2003).

When fungi are present in a given environment, they are generally in great numbers and it is almost impossible to avoid microfungi. Exposure to microfungi generally does not have a strong effect but these organisms have a potential to effect human health either direct or indirect ways (Stark et al. 2005). *Alternaria* and *Cladosporium* are known to be the most important allergens present in outdoor air, whilst *Aspergillus* and *Penicillium* were reported recently to be the fungal allergens important in indoor air (Fischer and Dott 2003).

It is well established that inhalation of fungal spores induces respiratory allergy symptoms in sensitized individuals; among all patients suffering from respiratory allergy symptoms, 20–30% of them are sensitized to fungi spores. These symptoms are clinically manifested as rhinitis, bronchial asthma, and alveolitis. Many authors correlate the onset of respiratory allergy symptoms with the presence of fungi spores in the atmosphere. It is well known that spores of different fungal species can be found in the atmosphere of a particular region representing a characteristic occurrence pattern for each particular region (Gioulekas et al. 2004).

Epidemiological surveys support that asthma affects 5–30% of children and 2–30% of adults. This wide range of inter-survey fluctuation is mainly due to the different geographic distribution and to the use of different criteria among studies. Researchers characterize it as “a chronic allergic disease of airways”. Rhinitis affects 40% of the population (Gioulekas et al. 2004).

Airborne dispersion is known to be the transportation way of agents of illness such as tuberculosis and aspergillosis. Airborne transportation is also responsible from nosocomial infections related to methicillin-resistant *Staphylo-*

coccus aureus (MRSA), *Acinetobacter* spp. and *Pseudomonas* spp. However, there are some doubts about the role of airborne type of transportation in nosocomial infections (Bernards et al. 1998; Beggs 2003).

Recent studies showed that the sensitivity to microfungi such as *Alternaria* is in close relation with allergic rhinitis and asthma in children (Stark et al. 2005).

Determination of bacterial and fungal concentrations in a hospital atmosphere is important not only in terms of the risk factors that they could cause but also the in terms of measures that should be taken on time. Patients being treated in hospital environments, especially children and the elderly, are generally immunocompromised persons and therefore are more sensitive to airborne bacterial and fungal pathogens. Thus, air control measures are important in terms of keeping the levels of indoor airborne biological particles in hospitals at desired low values. Since a relation between nosocomial outbreaks and pathogens was found, air sampling is recommended for epidemiological approaches especially when an epidemic case appears (Gangneux et al. 2006).

Determination of bacterial and fungal concentrations in a hospital environment is an important means in foreseeing the possible risks they might cause and lead hospital managements to apply measure policies. Nosocomial infections can occur in every service and in every age group in a hospital, but children, as well as old patients, and patients with chronic sickness constitute the most risky group. The morbidity and mortality seen in infections in pediatric services are known to be in high levels. Among young patients, premature babies and newborns are most vulnerable to nosocomial infections (Gürler 2004).

It is clear that children and the elderly are more sensitive to airborne bacterial and fungal pathogens and determination of potential pathogens present in air is of major importance to keep these individuals' health. In an air sampling study, humidity, temperature, and pressure values must also be recorded to reveal the effects of these parameters on bacterial and fungal concentrations.

Materials and methods

Sampling sites and sampling procedure

Samples were taken from either indoor or outdoor atmosphere of the Pediatric Service and Polyclinic of the Edirne Government Hospital in the city of Edirne. Some characteristics of the sampled sites were given in Table 1.

Sampling of culturable airborne microfungi and bacteria were done using the Petri plate gravitational settling method. Samples were collected at 1-month intervals over a period of 12 months between January 2004 and December 2004. Petri plates were exposed to air 50–80 cm above ground level for every 10-min indoor sampling session, while outdoor air samples were collected at a height of 150 cm above ground surface for 15 min. This sampling method has been widely used in monitoring studies in both indoor and outdoor environments (for details, see Abdalla 1988; Atik 1993; Şimşekli 1994; De-Wei and Kendrick 1995).

Peptone dextrose agar containing Rose bengal and streptomycin was used to catch, enumerate, and isolate the fungi in the air. Rose bengal stain was added to limit the growth of fast-growing moulds and streptomycin antibiotic was supplemented to control the reproduction of bacteria in the medium. The plates were incubated at 25°C for 7–10 days; growing fungi were counted at the end of the incubation period (results reported as the number of colony forming units—CFU) and

subcultured on slant potato dextrose agar medium in tubes before identification.

Bacteria were collected on brain–heart infusion agar (with 5% sheep blood). Blood-based nutrients are nonselective culture media that are widely used for broad-spectrum studies. Culture plates were incubated under aerobic conditions for 24–48 h at 37°C. Colonies on each plate were counted and bacterial results were reported as CFU)

Identification

Identification of the fungi was based on their macro- and microscopic features. The determination of micro morphological structures of fungi was carried out on material mounted in a modified mounting medium, Lacto-Cotton Blue, proposed by Sime et al. (2002). Barnett and Hunter (1999) work provided the basis for the identification of fungi at the genus level. Pitt (1979, 2000), Samson et al. (2002), Samson and Pitt (2000) were used for *Penicillium* species. *Penicillium* cultures were inoculated for identification in triplicate on three different media (Czapek yeast autolysate agar (CYA), malt extract agar (MEA) and 25% glycerol nitrate agar) instructed as detailed by Pitt (1979, 2000) and incubated at three different temperatures (5, 25, and 37°C). *Aspergillus* species were identified according to the monographs by Raper and Fennell (1965), Klich (2002), and Samson et al. (2002). Czapek solution agar, CYA,

Table 1 Some characteristics of indoor and outdoor sampling sites giving data on conditioning, heating, contamination sources, people densities and vegetation types

	Sampling sites				
	Indoor				Outdoor
	Service		Polyclinics		
	Room	Corridor	Room	Corridor	
Air conditioning	No device, only with windows	No device, windowless	No device, only with windows	No device, windowless	–
Heating	Radiators	Radiators	Radiators	Radiators	–
Possible sources of contamination	Vegetation, patients and their attendants in the room, food and flowers coming outside	Peoples, vegetation grown in flowerpots and the soil used	Doctors, nurses and patients	Patients	–
Inhabitant density	2–9	Dense	2–4	Rather crowded	–
Vegetation types	Minor	Minor	–	–	Diverse

MEA, Czapek yeast extract agar with 20% sucrose media were used for the identification of *Aspergillus* species. “*Fusarium* species—An Illustrated Manual for Identification” (Nelson et al. 1983) was used for the identification of *Fusarium* species-inoculated PDA plates. Other genus and species-inoculated PDA and MEA plates were identified according to Ellis (1971), Ellis and Ellis (1997), Samson et al. (2002), and Hasenekoglu (1991). Fungal author names, as cited in this paper, are standardized in accordance with Authors of Fungal Names (Kirk and Ansell 1992) and the website, www.indexfungorum.org. List of accepted species and synonyms in the family Trichocomonaceae (Pitt 2000) is followed for acceptability names of *Penicillium* and *Aspergillus* species.

Bacterial identification was based primarily on morphology, Gram staining, and endospore formation. Bacteria were grouped as Gram (+) cocci, Gram (+) bacilli, endospore-forming Gram (+) bacilli and Gram (–) bacteria, according to their microscopic morphology. Some commonly found bacteria were identified at the genus level using conventional methods according to Baron et al. (1994), Howard et al. (1987), and Holt et al. (1994). These methods were based on biochemical reactions of bacterial samples, i.e., TSI, DNase test, oxidase test, and further microscopic investigations of the Gram-stained preparations.

Meteorological measurements

Monthly and sampling day average measurements of meteorological parameters such as temperature, relative humidity, wind velocity, rainfall, and sunny time were supplied from the Directorate of Edirne Meteorological Office during the course of our study (Table 2). Indoor air temperature and relative humidity were also monitored using a thermometer and hygrometer (TFA-Dostmann GmbH, Germany) at the study sites (Table 3).

Statistical analysis

Correlation analyses were performed to determine the relationships between meteorological factors and viable spore counts of microfungi genera and bacteria isolated over the 12 months. Mann–Whitney *U* Test, ANOVA test, and Kruskal–Wallis analysis were used to compare the differences and similarities among sampling sites *p* values of <0.05 were considered as significant. Also, the ANOVA test and Kruskal–Wallis analysis was performed to compare indoor and outdoor air of sampling sites in terms of bacterial and microfungi genera found throughout the 12-month period.

Temperature and humidity values of each station were measured and recorded during the whole study period. A correlation analysis was

Table 2 Some meteorological measurements obtained monthly throughout the study period

Months	Temperature (°C)		RH (%)		Rain (mm)		Wind speed (m/sn)		Sunny period (hour/day)	
	M	D	M	D	M	D	M	D	M	D
January	2.2	8.9	80.1	93	61.2	4.1	2.1	2.6	2.1	0.0
February	4.9	2.2	70.7	66.3	9.4	0.0	2.3	0.5	4.0	9.0
March	8.6	11.5	68.8	62.3	34.1	0.0	2.0	1.0	4.9	6.5
April	13.2	12.0	59.8	71.0	14.6	5.3	2.1	2.1	5.9	3.3
May	17.1	13.6	62.8	80.3	59.6	10.3	1.8	1.3	8.2	1.8
June	22.1	19.6	68.9	81.3	95.2	5.8	1.4	1.2	8.4	3.9
July	24.6	23.8	59.1	57.0	34.1	0.0	1.7	2.4	11.2	13.8
August	24	20.7	62.0	58.7	25.1	0.0	1.5	2.3	10.1	9.9
September	20.6	21.7	62.4	64.3	0.5	0.0	1.8	1.0	8.4	8.1
October	16.5	18.9	70.6	79.3	5.2	2.1	1.5	1.9	5.3	0.6
November	9.8	1.5	75.6	62.3	27.8	0.0	1.4	1.4	3.9	8.5
December	5.5	13.7	82.5	81.0	107.1	0.0	1.8	3.9	2.8	2.5
Mean	14.1°C		68.6%		39.5 mm		1.8 m/sn		6.3 h/day	

RH relative humidity, M monthly, D daily

Table 3 Indoor environmental properties (temperature (°C) and relative humidity (%) values) that were measured in the January 2004–December 2004 indoor air (with TFA-Dostmann GmbH, Germany)

Months		Service										Polyclinics		
		A	B	C	D	E	F	G	H	K ^a	L ^a	M	N	P ^a
January	°C	24	22	25	25	25	27	25	25	11	11	24	23	11
	R.H %	74	74	74	74	74	74	74	74	80	80	73	73	80
February	°C	24	25	25	24	24	25	24	23	2	2	22	23	2
	R.H %	62	62	62	63	63	62	63	63	63	63	65	64	63
March	°C	23	23	24	24	23	23	23	24	15	15	24	23	15
	R.H %	33	33	33	33	33	33	33	34	44	44	35	34	44
April	°C	23	23	23	23	22	22	22	22	13	13	22	21	13
	R.H %	45	44	44	45	46	44	44	44	35	35	46	46	35
May	°C	21	21	21	21	21	21	21	21	16	16	23	22	16
	R.H %	55	53	54	54	53	53	54	52	62	62	57	56	62
June	°C	25	25	25	25	24	25	24	24	27	27	30	27	27
	R.H %	47	47	48	47	49	44	49	51	55	55	44	44	55
July	°C	28	28	28	28	28	28	28	28	29	29	28	28	29
	R.H %	37	37	38	38	37	37	37	38	32	32	35	35	32
August	°C	27	27	28	27	28	27	28	27	27	27	28	29	27
	R.H %	58	58	58	58	58	57	58	57	54	54	52	54	54
September	°C	26	25	25	25	25	25	26	25	26	26	26	27	26
	R.H %	55	55	55	55	55	55	55	55	57	57	55	54	57
October	°C	25	25	24	24	24	24	24	24	25	25	24	24	25
	R.H %	60	59	59	58	59	59	58	60	60	60	57	57	60
November	°C	24	24	23	23	24	24	24	22	6	6	20	20	6
	R.H %	27	27	25	25	26	26	26	27	20	20	31	29	20
December	°C	24	24	23	23	23	23	22	22	14	14	21	21	14
	R.H %	68	68	67	67	67	67	66	66	52	52	69	69	52

°C Temperature, R.H % relative humidity (%)

^aOutdoor

performed in order to determine presence of a relation between the meteorological factors in question, isolated bacterial and fungal genera and their total concentrations. A Mann–Whitney *U* test was performed to evaluate the differences between polyclinics and service in terms of their indoor and outdoor airs, and Mann–Whitney *U* test and Kruskal–Wallis analysis were performed to evaluate the differences between total colonial numbers and seasons. $p < 0.05$ was used as a significant value.

Results

The Petri plate gravitational method applied allowed us to evaluate indoor and outdoor bacterial and microfungi colonial counts in different sampling stations in a hospital environment dur-

ing different seasons. The results obtained were evaluated with respect to sampling months, stations and seasons. All sampled colonies, either bacterial or microfungi, were identified based on conventional methods. The identified species were given in Table 6. The monthly distributions of microfungi and bacteria isolated from both indoor and outdoor airs of 13 stations showed that a total of 1,376 (912 service, 464 polyclinic) microfungi and 2,429 (1,748 service, 681 polyclinic) bacterial colonies were present (for details see Table 4). The number of microfungi colonies in indoor air (782 colonies; 56.83%) appeared to be higher when compared to outdoor air (594 colonies; 43.16%; Tables 4 and 5).

We found that August was the leading month with 374 (27.18%) microfungi colonies, followed by May with 236, June with 171, July with 159, October with 102, January with 64, November

Table 4 Total monthly colonial numbers in terms of CFU isolated from all stations

Months		Service								Outdoor		Polyclinics			Total
		Indoor								Outdoor		Indoor		Outdoor	
		A	B	C	D	E	F	G	H	K	L	M	N	P	
January	B	10	6	15	11	11	13	17	17	6	11	31	13	6	167
	M	–	1	–	1	1	–	33	4	6	5	5	2	6	64
February	B	21	6	16	38	53	1	8	25	7	19	17	23	21	255
	M	–	–	1	1	1	–	1	–	3	7	11	5	3	33
March	B	16	12	20	22	19	3	11	19	18	15	3	6	34	198
	M	–	–	1	1	1	–	–	2	6	10	3	–	4	28
April	B	14	4	20	26	35	7	14	4	16	21	10	21	32	224
	M	2	1	1	3	9	1	3	–	2	12	3	–	13	50
May	B	18	3	18	11	6	6	6	17	8	6	4	9	16	128
	M	19	8	12	13	8	5	16	9	20	26	31	29	40	236
June	B	8	4	2	13	10	3	4	3	15	27	10	9	23	131
	M	6	10	5	7	12	7	11	14	18	35	8	5	33	171
July	B	7	18	19	16	12	32	5	8	26	42	11	8	30	234
	M	7	9	12	15	10	12	10	2	16	17	5	7	37	159
August	B	16	4	10	4	10	10	10	13	22	10	24	10	47	190
	M	19	20	23	25	27	10	12	10	43	35	32	54	64	374
September	B	12	5	22	11	12	12	18	13	28	24	12	7	19	195
	M	5	2	2	2	–	1	3	9	3	13	2	1	3	46
October	B	21	16	13	28	16	6	17	11	28	20	12	11	35	234
	M	7	4	3	5	3	5	4	13	21	17	5	4	11	102
November	B	6	7	36	22	47	3	8	12	31	34	23	38	30	297
	M	–	4	2	–	1	–	4	–	15	13	3	6	12	60
December	B	16	6	3	16	3	6	2	3	22	23	32	27	17	176
	M	4	1	1	6	3	2	1	2	9	7	4	4	9	53
Total	B	165	91	194	218	234	102	120	145	227	252	189	182	310	2429
	M	69	60	63	79	76	43	98	65	162	197	112	117	235	1376
Indoor–outdoor total	B	1269								479		371		310	
	M	553								359		229		235	

The data belongs to the period between January 2004 and December 2004

*B/M*bacteria/microfungi. *A* service corridor, *B* nurse's room, *C* nine-bed room, *D* four-bed room, *E* four-bed room, *F* incubator room, *G* three-bed room, *H* two-bed room, *K* outdoor facing station D, *L* outdoor facing station H, *M* polyclinic, *N* polyclinic corridor, *P* polyclinic outdoor)

Table 5 Monthly outdoor and indoor fungal colonies

	Indoor	Indoor %	Outdoor	Outdoor %
January	47	6.01	17	2.86
February	20	2.56	13	2.19
March	8	1.02	20	3.37
April	23	2.94	27	4.55
May	150	19.18	86	14.48
June	85	10.87	86	14.48
July	89	11.38	70	11.78
August	232	29.67	142	23.91
September	27	3.45	19	3.20
October	53	6.78	49	8.25
November	20	2.56	40	6.73
December	28	3.58	25	4.21
Total	782		594	

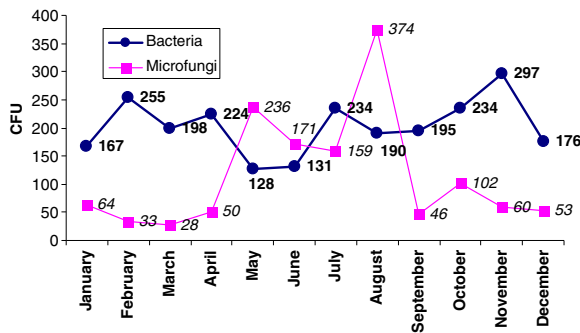


Fig. 1 The presence of airborne bacteria and microfungi, in terms of CFUs, for each month of the study period. The colonial counts for indoor and outdoor samples for each month was given together

with 60, December with 53, April with 50, September with 46, February with 33 and March with 28 colonies (Fig. 1).

When the results were evaluated regarding the bacterial numbers this time November was determined to lead with 297 (12.22%) colonies followed by February with 255, July and October with 234, April with 224, March with 198, September with 195, August with 190, December with 176, January with 167, June with 131, and May with 128 colonies (Fig. 1).

When isolated colonies were evaluated regarding their presence in service and polyclinics, it appeared that 1,748 (67.7%) of 2,660 colonies isolated from service were bacterial and 912 (34.3%) were microfungal colonies and that 681 (59.4%) of 1,145 colonies isolated from polyclinics were bacterial and the remaining 484 (40.6%) were microfungal (Figs. 2 and 3).

The identifications of isolated samples revealed that microfungi were represented with 66 species from 16 genera. *Cladosporium* was found to be the most abundant genus with 462 colonies (33.58%)

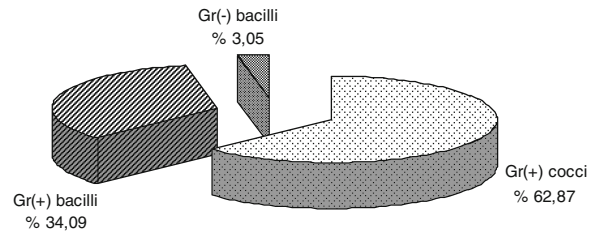


Fig. 2 Percentage distributions of bacterial types isolated between January 2004 and December 2004

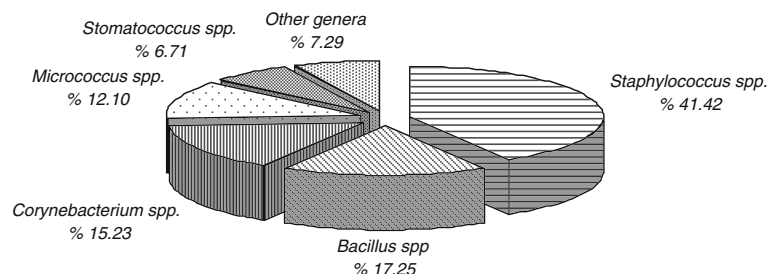
followed by *Cladosporium* and *Alternaria* with 310 (22.53%), *Penicillium* with 280 (20.35%), *Aspergillus* with 66 (4.8%), and *Acremonium* with 52 colonies (3.78%; Fig. 4).

During the study period, common isolated fungi for all months were *Cladosporium*, *Alternaria*, and *Penicillium* (Fig. 4). Among the most common microfungi genera, *Cladosporium* and *Alternaria* was most abundant during August with 107 and 204 colonies, respectively and *Penicillium* was most abundant during May with 47 colonies.

When the results obtained for microfungi were evaluated in terms of species diversity, *Penicillium* was found to be the most diverse genus with 22 species, followed by *Cladosporium*, *Aspergillus*, and *Alternaria* with 13, 8, and 4 species, respectively (Table 6). *Cladosporium cladosporioides* was the most common isolated species by being represented during 9 months of the study period.

Summer season was found to be the season when microfungi were isolated in highest numbers (704 colonies) followed by spring (314 colonies), autumn (208 colonies), and winter (150 colonies). Among the genera isolated during summer, *Alternaria* was represented with 255 colonies whereas *Cladosporium* and *Penicillium* were represented with 242 and 50 colonies, respectively

Fig. 3 Percentage distributions of bacterial genera isolated between January 2004 and December 2004



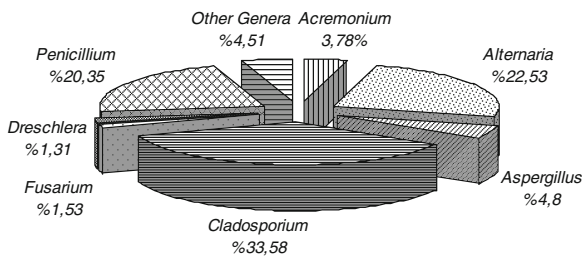


Fig. 4 The microfungi genera isolated during the study

(Fig. 5). In contrast to the summer season, *Cladosporium* was represented with 126 colonies in spring followed by *Cladosporium* and *Alternaria* with 69 and 31 colonies, respectively.

Isolated bacteria were grouped into three categories regarding their Gram-staining properties and the results showed that Gram (+) cocci were

the most abundant group with 1,527 colonies (62.87%) followed by Gram (+) bacilli with 828 (34.09%) and Gram (–) bacilli with 74 colonies (3.05%). Gram (–) cocci were not isolated during any time of the study period (Fig. 3); 419 sporulated Gram (+) bacilli colonies were determined constituting 50.60% of annual Gram (+) bacilli.

The dominant group in indoor air was represented with Gram (+) cocci (74.88%) and with Gram (+) bacillus (60.20%) in outdoor air.

Isolated colonies were identified and a total of 15 genera were determined among which *Staphylococcus* was the most abundant one with 1,006 colonies (41.42%) followed by *Bacillus* with 419 (17.25%), *Corynebacterium* with 370 (15.23%), *Micrococcus* with 294 (12.10%), and *Stomatococcus* with 52 (6.71%) colonies (Fig. 4). *Staphylococcus*, *Bacillus*, *Corynebacterium*, and *Micrococcus*

Table 6 Isolated microfungi species, the months during which they were isolated and their colony numbers (the numbers in brackets correspond to the colony numbers for each species)

Species	Isolation month and colony numbers
<i>Acremonium kiliense</i> Grütz	5 (6), 7 (1)
<i>Alternaria alternata</i> (Fr.) Keissler	1 (4), 3 (1), 5 (12), 7 (23), 8 (116), 9 (8)
<i>A. citri</i> Ellis & N. Pierce	4 (2), 7 (3), 8 (21), 9 (11)
<i>A. dianthi</i> Stevens & Hall	7 (5), 8 (17)
<i>A. longipes</i> Ellis & Everh	6 (2)
<i>A. tenuissima</i> (Kunze ex Pers)	5 (13), 6 (8), 7 (3), 8 (50), 9 (1)
<i>Aspergillus flavus</i> Link	10 (8)
<i>A. fumigatus</i> Fresen	3 (1), 6 (4), 7 (3), 10 (4), 11 (2)
<i>A. niger</i> Van Tieghem	3 (3), 4 (2), 6 (2), 9 (1), 10 (2), 11 (2), 12 (1)
<i>A. parasiticus</i> Speare	4 (3)
<i>A. sclerotiorum</i> Huber	1 (3)
<i>A. terreus</i> Thom	5 (7), 6 (2)
<i>A. versicolor</i> (Vuill.) Tiraboschi	9 (2), 10 (3), 11 (2)
<i>A. wentii</i> Wehmer	4 (5), 7 (4)
<i>Cladosporium aecidicola</i> Thüm	4 (2), 5 (3), 7 (2), 8 (2), 9 (1)
<i>Cladosporium apicale</i> Berk. & Br.	9 (2)
<i>C. chlorocephalum</i> (Fresen.)	1 (3), 2 (1), 4 (1), 5 (3)
E. W. Mason & M. B. Ellis	
<i>C. cladosporioides</i> (Fresen.) De Vries	3 (2), 5 (5), 6 (22), 7 (3), 8 (62), 9 (4), 10 (13), 11 (3), 12 (5)
<i>C. cucumerinum</i> Ellis & Arthur	3 (3), 5 (8), 6 (7), 8 (6), 9 (1), 10 (1), 11 (3), 12 (4)
<i>C. elatum</i> (Harz) Nannf.	5 (6), 6 (3), 7 (17), 8 (12), 10 (8)
<i>C. herbarum</i> (Pers.) Link	1 (2), 3 (2), 5 (13), 6 (38), 7 (8), 8 (19)
<i>C. macrocarpum</i> Preuss	5 (5), 9 (1), 10 (10)
<i>C. oxysporum</i> Berk. & Curt.	5 (1)
<i>C. sphaerospermum</i> Penz	3 (3), 5 (15), 6 (14), 8 (6), 9 (1), 11 (17), 12 (5)
<i>C. spongiosum</i> Berk. & Curt.	2 (1), 5 (2)
<i>C. tenuissimum</i> Cooke	4 (2), 5 (17), 6 (10), 7 (11), 10 (12)
<i>C. variabile</i> (Cooke) De Vries	4 (5), 5 (26), 9 (2)
<i>Camarosporium lauri</i> (Sacc.) Grove	3 (1)

Table 6 (continued)

Species	Isolation month and colony numbers
<i>Drechslera australiensis</i> M. B. Ellis	7 (10)
<i>D.ellisi</i> Danguah	7 (5)
<i>D. hawaiiensis</i> Subram& Jahex M.B.Ellis	7 (1)
<i>Fusarium concolor</i> Reinting	8 (10)
<i>F.oxysporum</i> Schlecht	8 (2)
<i>F.trichothecioides</i> Wollenw.	8 (2)
<i>Penicillium aurantiogriseum</i> Dierckx	2 (1)
<i>P. bilaii</i> Chalabuda	4 (1)
<i>P. brevicompactum</i> Dierckx	5 (8), 11 (3)
<i>P. chrysogenum</i> Thom	3 (3), 5 (6), 10 (1), 11 (6), 12 (7)
<i>P. citrinum</i> Thom	1 (35), 5 (12), 10 (3), 11 (2)
<i>P. crustosum</i> Thom	4 (12), 8 (14), 12 (3)
<i>P. digitatum</i> (Pers.: Fr.) Sacc.	10 (4)
<i>P. expansum</i> Link	11 (5)
<i>P. fellutanum</i> Biourge	2 (7), 11 (1), 12 (5)
<i>P. glandicola</i> Seifert & Samson	7 (8), 11 (5), 12 (8)
<i>P. glabrum</i> Wehmer	4 (1)
<i>P. griseofulvum</i> Dierckx	1 (1)
<i>P. herquei</i> Bain & Sartory	5 (12)
<i>P. italicum</i> Wehmer	3 (1), 6 (11), 12 (4)
<i>P. lividum</i> Westling	2 (7), 10 (1), 12 (3)
<i>P. olsonii</i> Bain & Sartory	10 (1), 12 (4)
<i>P. raistrickii</i> G.Smith	10 (1)
<i>P. roquefortii</i> Thom	5 (9), 11 (1)
<i>P. verrucosum</i> Dierckx	10 (7), 11 (2)
<i>P. verruculosum</i> Peyronel	9 (4)
<i>P. viridicatum</i> Westling	2 (5), 3 (1), 4 (1), 7 (4), 11 (2)
<i>P. waksmanii</i> K.M. Zalesky	6 (4), 10 (16)
<i>Phoma glomerata</i> (Corda) Wr.& Hochapfel	1 (3), 3 (5)
<i>Scopuloriopsis brevicaulis</i> (Sacc.) Bain	2 (1), 6 (1)
<i>S. flava</i> (Sopp) Morton & G. Smith	5 (1)
<i>Trichoderma viride</i> Pers. Ex Gray	7 (1), 9 (2)
<i>Trichothecium roseum</i> (Pers.) Link	12 (2)
<i>Ulocladium alternariae</i> (Cooke) Simmons	11 (2)
<i>U. atrum</i> Preus	12 (2)
<i>U. botrytis</i> Preus	4 (1)
<i>Verticillium funicola</i> (Preus)Hassebrauk	5 (1)

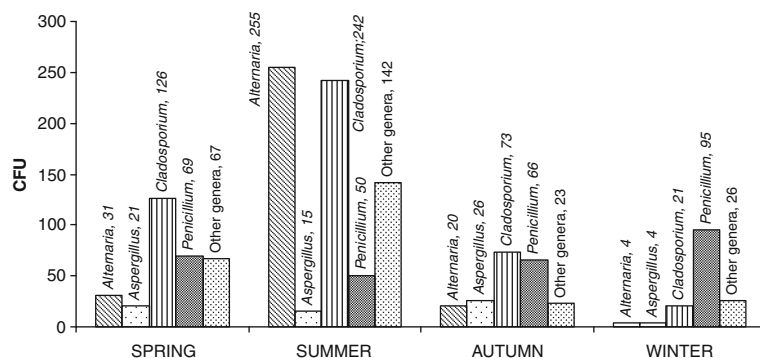
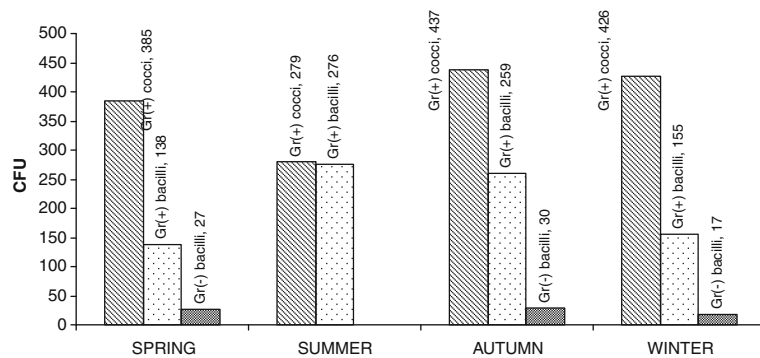
Fig. 5 Seasonal distributions of isolated microfungi in terms of colony numbers


Fig. 6 Seasonal distributions of isolated bacteria



were found to be the genera isolated commonly during all months.

When isolated bacteria were grouped according to their seasonal abundance, autumn was found to be the season during which the highest colonial numbers were isolated (726 colonies), followed by winter with 598, summer with 555, and spring with 550 colonies. Among the colonies isolated during autumn, Gram (+) cocci were represented with 426 colonies, Gram (+) bacilli with 155 colonies, and Gram (-) bacilli with 30 colonies (Fig. 6).

Discussion

This present study was carried out to determine the airborne microfungal and bacterial contents of indoor and outdoor atmosphere of Pediatric Service and Policlinic of Edirne Government Hospital. The isolated microfungi colonies were identified using standard biochemical and microscopic methods and the following pattern was obtained. The present data on what we obtained about the types and concentrations of microfungi is similar to those revealed by a number previous indoor air studies (Katz et al. 1999; Gorny and Dutkiewicz 2002; Sarica et al. 2002; Aydogdu and Asan 2008; Martins-Diniz et al. 2005). The most frequently isolated microfungi in all sampled stations was *Cladosporium*, irrespective of being indoor or outdoor, with 462 colonies comprising 33.58%. *Cladosporium* was followed by 310 *Alternaria* (22.53%) and 280 *Penicillium* (20.35%) colonies. This ordering for fungal colonial counts was also found in similar studies performed in

Turkey (Asan et al. 2002; Sen and Asan 2009; Suerdem and Yildirim 2009).

We found a significant difference between the total number of fungi isolated in stations with respect to season of the year ($p < 0.001$). As expected, the number of isolates was significantly more in summer than in winter ($p < 0.001$). This is also the case in most of other studies. A temperature between 15 and 25 °C and a 60–70% relative humidity led to an increase in outdoor airborne fungal spore concentrations (Pepelnjak and Segvic 2003). It is most probably that heavy rains, low temperature, or dry air (in indoor conditions) account for the decrease in fungal spore numbers in winter.

The data we recorded during the study period allowed us to determine the type of relation between fungal spores and climatological factors. In contrast to the findings of previous studies reporting a positive correlation between fungal spore concentrations and wind, we determined, in two of the outdoor sampling stations (stations K and L), a negative correlation between fungal numbers and mean monthly wind parameters (station K; $r = -0.688$, $p = 0.013$ —station L; $r = -0.668$, $p = 0.018$). Fungal spore concentrations decreased with increasing wind. This wind effect for decreasing colonial counts can be attributed to the high wind velocity which most probably prevented spores to settle down on the plates we exposed to air.

A correlation analysis revealed a positive correlation between *Cladosporium* and mean monthly temperature, meaning that the concentration of isolated *Cladosporium* spores increased with increasing atmosphere temperature ($p = 0.014$). As

a result of this positive correlation, spring and summer months were the months during which *Cladosporium* (and also *Alternaria*) was isolated in big numbers. Fungi with spores similar to those of *Cladosporium*, *Alternaria*, *Epicoccum*, and *Dreschlera* intend to exist in higher concentrations in dry and hot weather conditions (Burch and Levetin 2002).

There exists evidence that *Penicillium* species can cause allergic reactions in some people. The statistical analysis revealed a positive correlation between *Penicillium* colony numbers and mean daily humidity value and daily rain amount ($p = 0.004$, $p = 0.042$). This means that *Penicillium* concentration increased with increasing rain and humidity. This positive correlation determined between can be explained by the fact that water is essential for fungal growth and dispersion. This also explains why *Penicillium* was determined more frequently in winter when the atmospheric humidity and rain was high. On the other hand, the presence of *Penicillium* in the atmosphere was found to be negatively correlated with mean daily sunny period ($p = 0.015$). Considering these results, we can conclude that *Penicillium* favors humid conditions and that they are less likely to be present in dry conditions.

Gangneux et al. (2006) performed a study in a hospital in Greece where patients of high risk were treated. Samples were taken from the atmosphere, from tap water, and from material surfaces in transplantation, hematology, and pediatric departments and child intensive care unit. The results of atmospheric sampling revealed that *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus* were present in indoor hospital air with concentration percentages of 25.9%, 17.7%, and 12.4%, respectively. We also isolated these three *Aspergillus* species in our present study. These species bear great importance in terms of possible health effects they might cause. For instance, these species, even when their concentrations are low, may lead to significant health hazards if they are present especially in rooms of immunocompromised child patients.

Among the sampled service rooms for their atmospheric bacterial contents, station E (the room with four patient beds) had the highest colony numbers with 234 colonies followed by station D

(also with four beds) and station C (with nine beds) with 218 and 194 colonies, respectively. This showed that, contrary to our expectations, the room with nine beds had the lowest bacterial numbers implying that surface area of a sampled place influenced the colonial numbers at least as the occupants there.

Gram (–) bacillus were shown to be the most frequent and significant health-threatening bacteria, but more recently a number of studies reported an increased isolation frequency for Gram (+) bacteria, mainly for CNS, *S. aureus* and enterococci (Çetin et al. 2007). In our study, *Staphylococcus* was found to be the most frequent bacterial genera with 1,006 colonies (41.42%). This genus was followed by *Bacillus* with 419 colonies (17.25%) and *Corynebacterium* with 370 colonies (15.23%). *Staphylococcus* is known to be the most common isolated genus from hospitals (Sarica et al. 2002; Pastuszka et al. 2005; Ekhaïse et al. 2008). *Staphylococcus* is known to be easily carried in the nasopharynx, throat, skin, cuts, boils, nails and as such can easily contribute to the microflora in the hospital environment (Ekhaïse et al. 2008).

All bacteria we determined in our study are common members of human flora and widely distributed in nature and are opportunistic pathogens. Dominant coagulase-negative staphylococci are resistant to unfavorable conditions and can live in room temperature for months.

Gram-positive bacteria isolated from the plates were predominantly of cocci in nature and include *Staphylococci* (lytic and nonlytic) and *Micrococci*. While earlier studies have proved *S. aureus* to be pathogenic, recent studies have shown that *Micrococci* (common hospital contaminants) and coagulase-negative *Staphylococci* are capable of causing a range of infections in immunocompromised patients (Sudharsanam et al. 2008). Gram (–) bacteria are generally present in low numbers but *Acinetobacter*, *Aeromonas*, *Flavobacterium* or especially *Pseudomonas* species can be common. The presence of these bacteria in a given place is an indication of the existence of a water supply there. We isolated much more Gram (+) bacteria than Gram (–) ones, for instance, *Acinetobacter* was isolated from four stations, showing that water was present in all these stations, making these

places vulnerable for further microbial contaminations

Therefore, determination of types and concentrations of airborne microorganisms in hospitals and clinics is of great importance. Studies on bioaerosols in hospitals, spread of airborne fungi and their control and epidemiological investigations of nosocomial infections may provide data for determination of biohazard procedures. These data also serve as quality control criterions. (Pastuszka et al. 2005).

Nosocomial infections increased during recent years and became an important cause of morbidity and mortality. Immunocompromised patients such as children and the elderly are under high risk in terms of such infections. Airborne microorganisms present in the air we inhaled in hospitals are one of the reasons for nosocomial infections. Therefore, it is necessary to be informed in detail about the epidemiology and pathogenesis of microorganisms responsible for nosocomial infections. Determination and identification of outdoor and indoor airborne microorganism in a hospital environment will provide a basis to achieve success for information gathering. Therefore, our study will serve as a step for further studies.

It is clear that more studies are necessary to investigate and learn more about the health effects of fungus and bacteria. According to Streifel et al. (1987) an air filter operating with recirculation of HEPA filters greatly reduced the mean fungal numbers. Considering our results and this fact, we suggest hospital managements to establish and use effectively a centralized air-conditioning system. Apart from this, we also suggest (1) use of surface disinfectants in hospital environments to prevent bacterial and fungal contaminations, (2) removal of wet organic materials from hospital environments, especially from rooms of immunocompromised patients, (3) development of a more useful infection control program for lengthened hospital monitoring, (4) use of air conditioners (without permanent water inside) made of materials resistant to biodegradation, (5) improvement in laboratorial services, use of effective and continuing disinfection procedures, education of the public on health topics and improvement in environmental conditions.

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