

Indoor airborne fungal pollution in newborn units in Turkey

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Abstract Pathogenic and/or opportunistic fungal species are major causes of nosocomial infections, especially in controlled environments where immunocompromised patients are hospitalized. Indoor fungal contamination in hospital air is associated with a wide range of adverse health effects. Regular determination of fungal spore counts in controlled hospital environments may help reduce the risk of fungal infections. Because infants have immature immune systems, they are given immunocompromised patient status. The aim of the present study was to evaluate

culturable airborne fungi in the air of hospital newborn units in the Thrace, Marmara, Aegean, and Central Anatolia regions of Turkey. A total of 108 air samples were collected seasonally from newborn units in July 2012, October 2012, January 2013, and April 2013 by using an air sampler and dichloran 18% glycerol agar (DG18) as isolation media. We obtained 2593 fungal colonies comprising 370 fungal isolates representing 109 species of 28 genera, which were identified through multi-loci gene sequencing. *Penicillium*, *Aspergillus*, *Cladosporium*, *Talaromyces*, and *Alternaria* were the most abundant genera identified (35.14, 25.40, 17.57, 2.70, and 6.22% of the total, respectively).

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Introduction

Fungi are common in indoor environments, and the indoor air quality of a hospital can affect patients and the staff who are in contact with them (Srinivasan et al. 2002; Araujo and Cabral, 2010; Méheust et al. 2013; Caggiano et al. 2014). The mycobiota of a hospital mainly consists of filamentous fungi, and this fungal air contamination produces fungal infections (Uztan et al. 2013). Exposure to airborne fungal particles and conidia may lead to respiratory diseases such as allergic rhinitis, asthma, and hypersensitivity pneumonitis

(Rainer et al. 2000; Wu et al. 2000; Munoz and Bouza 2001; Singh and Paterson 2005; Vackova et al. 2006). Infections with filamentous fungi such as aspergillosis and mucormycosis are serious for patients, and these infections are increasing worldwide (Caggiano et al. 2014). This leads to an increase in the mortality rate and the cost of the patient care (Dessi 2014). The cost of treatment of nosocomial infections caused by *Aspergillus* is high, and the mortality rate is usually over 50% (Vackova et al. 2006). *Aspergillus fumigatus* is a very important airborne nosocomial fungal pathogen in hospital environments, and it can cause severe mycosis (Desoubeaux et al. 2014). Other fungi such as *Zygomycetes* members may affect particularly susceptible hosts (Garnacho-Montereo and Amaya-Villar 2006; Perlroth et al. 2007; Dagenais and Keller 2009; Shoham and Marwaha 2010).

Newborns, especially those born prematurely, have undeveloped immune systems and are consequently prone to infections. Fungal pathogens are important risk factors for invasive disease together with other factors such as prematurity, usage of central venous catheters, histamine blockers and antibiotics, intubation, and prolonged length of hospitalization (Rowen 2001; Manzoni et al. 2012; Ellsworth et al. 2012; Hundalani and Pammi 2013). During the past two decades, invasive fungal infections (IFIs) have become an increasingly important problem in preterm infants hospitalized in the Neonatal Intensive Care Unit (NICU) (Montagna et al. 2010; Alangaden 2011; Méheust et al. 2014). The morbidity and mortality rates caused by fungal infections in pediatric services are high. In particular, young patients, premature babies, and newborns are most vulnerable for nosocomial infections in these services (Okten and Asan 2012).

Recently, many studies have attempted to evaluate the importance of obtaining information about airborne opportunistic pathogens as a measure of preventing hospital acquired infections (Gangneux et al. 2006; Araujo and Cabral 2010; Caggiano et al. 2014). Sarica et al. (2002) conducted a study in different parts of Trakya University Hospital containing air of birth room and determined some airborne fungi such as *A. fumigatus* and *Aspergillus niger* by classical identification methods such as morphological and colonial properties of fungi. At this time, we have limited information about fungal contaminant types and their biodiversity in newborn intensive care units, especially in Turkey.

The purpose of this study was (i) to describe the types and relative frequency of culturable airborne fungi in indoor air from five different hospital newborn units in the Thrace, Marmara, Aegean, and Central Anatolia regions of Turkey in different seasons of the year; (ii) to compare the species distribution in newborn intensive care units; and (iii) to evaluate mycological air quality of investigated hospitals to inform public health control for the protection of human health.

Materials and methods

Sampling areas

Our study was conducted in the NICUs of medical faculties of University training hospitals of Trakya (Edirne city), Istanbul (Istanbul city), Eskisehir Osmangazi (Eskişehir city), Ege (Izmir city), and Celal Bayar (Manisa city) Universities in respectively the Thrace, Marmara, Aegean, and Central Anatolia regions of Turkey (Fig. 1). Air sampling was performed in July 2012, October 2012, January 2013, and April 2013 on levels 1, 2, and 3 care units, a corridor, a milk storage room, and an isolation room.

Air sampling

Air samplers use volumetric impaction (an active sampling method) for fungal spore isolation. As described by Andersen Device (Andersen 1958; Araujo and Cabral 2010), air is aspirated through a grid perforated with a pattern of 286 calibrated holes. Air sampling was performed using three different sampling devices with the same properties and working principles, which were calibrated prior to the beginning of the study [Merck Mas 100 Eco (for Izmir and Manisa Cities), Biomerieux Air IDEAL (for Eskisehir City), Millipore M Air-T (for Edirne and Istanbul Cities)]. The air samples were taken in 3-month intervals. The middle months of each season were selected in order to represent every seasons' climatological data. Taking into consideration the square meters of sampling areas, 100 L of air was taken in duplicate (total 200 L) from each sampling area between 11:00 a.m. and 13:00 p.m. The air samplers were located in the center of the rooms away from open windows and doors at 1.40 m from ground level to match the breathing height of humans.



Fig. 1 A map of Turkey showing the research stations for the current study: Trakya University Medical Faculty at Edirne (Thrace region), Istanbul University Istanbul Medical Faculty at Istanbul (Marmara region), Ege University Medical Faculty at

Izmir, University Medical Faculty at Manisa (Aegean region), and Eskişehir Osmangazi University Medical Faculty at Eskişehir (Central Anatolia region)

Isolation of air fungi

Dichloran 18% glycerol agar (DG18) (Merck 1.00465) was used as an isolation medium. The number of CFUs for culturable airborne fungi was counted after 5 days of incubation at 25 °C for determination and investigation of fungal pollution and 37 °C for investigation of thermotolerant strains. The colony forming units per cubic meter were calculated (CFU/m³) (EL-Morsy 2006). Pure cultures were obtained using the single spore isolation method (Choi et al. 1999). The isolates were transferred to slants containing Potato Dextrose Agar (PDA) (Merck 1.10130) and Malt Extract Agar (MEA) (Merck 1.05398) and stored at +4 °C.

Measurements of temperature and relative humidity

Temperature and humidity levels of sampling areas were measured by using a Digital Thermo-Hygrometer (TFA 30.5002; indoor temperature −10 and +60 °C, accuracy ±1 °C (±1.8 °F) at −10 and +50 °C (+14 + 122 °F), humidity 10 and 99% rH, accuracy ±5% at 25–75% rH at 0–50 °C (+32 and +122 °F)) device.

Morphological and multi locus gene identification

Isolated fungi were initially identified to genus level according to their microscopic and colonial characteristics (Samson et al. 2004, 2010, 2014). For molecular identification, isolates were grown on MEA for 7 days at 25 °C before DNA extraction. DNA was extracted from the cultures using the ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research) following the manufacturer's protocols.

Molecular characterization of the isolates was performed using sequencing of the standard gene regions which are internal transcribed spacer (ITS) regions of the rDNA genes, part of the β -tubulin (*BenA*), calmodulin (*CaM*), and actin genes. The ITS regions and *BenA* genes were amplified using described methods (Visagie et al. 2014). For *CaM*, the CL1 and CL2 primer sets were used and for actin, the ACT-512F and ACT-783R primer sets were used (Carbone and Kohn 1999; Serra et al. 2006).

PCRs were performed using a Veriti® 96-Well Thermal Cycler (Applied Biosystems®) using described methods (Visagie et al. 2014). PCR products were separated by agarose gel electrophoresis (1% w/v in 1xTAE) and visualized by GelRed staining. PCR products were isolated using EXOSAP-IT (Affimetrix) and used for sequencing. Sequencing reactions were performed with the CEQ™ DTCS Quick Start Kit (Beckman Coulter) or Seq.Enzyme (BigDye 3.1) and sequenced with the CEQ 8000 Genetic Analysis System or the 3730 XL DNA Analyzer.

Data analyses

SeqMan [SeqMan Pro v. 9.0.4 (DNASTAR)] was used for assembling DNA sequences and alignments were performed using the Muscle in MEGA 6.0 program (Tamura et al. 2013). Maximum composite likelihood (MCL) analysis as performed by the MEGA 6.0 program was used for investigation of positions on phylogram with 1000 bootstrap replications. The obtained sequence data have been deposited in GenBank with accession numbers.

The Kruskal–Wallis analysis was used to test for statistical differences between sampling area and seasons in temperature, humidity, and total airborne fungi amount. In all analyses, P values are described as $P = 0.05$. In addition, Spearman correlation coefficients between concentration of airborne fungi (CFU/m³), temperature (°C), and relative humidity (%) were calculated. All statistical analyses were conducted using the software program SPSS version 24.0 for Windows (IBM).

Results and discussion

The objectives of this study were to determine the concentration, distribution, and identification of airborne fungal pollution in the newborn units of five different hospitals in the Thrace, Marmara, Aegean, and Central Anatolia Regions of Turkey. During air

sampling, mean temperature and mean relative humidity were between 19.6–31.4 °C and 17–64.7%, respectively (Table 1). The Kruskal–Wallis test showed statistically significant seasonal differences for airborne fungi amount in sampling seasons (Kruskal–Wallis test, $X^2 = 27.984$, $p = 0.000$). When considering the seasonal levels of fungal pollution, the highest fungal pollution were recorded at October 2012 (average 263.15 CFU/m³) and the lowest fungal pollution occurred in April 2013 (average 83.52 CFU/m³) (Fig. 2a). The results of the Kruskal–Wallis test indicated that there were no statistically significant differences between the numbers of fungal density recovered from sampling points (Kruskal–Wallis test, $X^2 = 3.333$, $p = 0.649$). However, the highest total fungal amount was calculated from inner corridor (average 208.75 CFU/m³), the lowest total fungal amount was calculated from level 2 care unit from among

Table 1 Temperature and humidity values in investigated hospitals

Hospital		Temperature (°C)						Humidity (%)					
		1	2	3	4	5	6	1	2	3	4	5	6
Trakya U. Hosp	Ju	31.0	31.0	30.0	28.0	31.0	31.0	37.0	36.0	38.0	35.0	37.0	36.0
	O	26.7	26.0	25.7	25.2	26.4	26.5	36.0	58.0	37.0	35.0	33.0	43.0
	Ja	24	25.1	22.1	25.4	25.0	25.4	37.0	34.0	36.0	33.0	31.0	36.0
	A	28.0	25.4	26.1	27.1	27.0	25.4	35.0	35.8	35.4	35.0	35.0	35.8
Istanbul U. Hosp.	Ju	31.4	31.3	30.7	31.8	32.3	30.7	55.9	51.0	53.6	51.4	57.5	64.7
	O	24.5	24.4	26.9	27.6	28.8	26.9	45.1	45.1	41.8	41.0	40.8	41.8
	Ja	22.6	21.9	23.7	25.6	25.0	24.4	49.8	56.2	44.5	35.5	38.3	42.6
	A	25.5	25.5	25.7	26.3	22.3	25.2	45.6	43.1	39.7	37.9	43.1	39.7
Osmangazi U. Hosp	Ju	28.7	27.2	28.2	27.5	28.0	28.0	30.0	33.0	32.0	35.0	31.0	56.0
	O	24.4	23.5	24.2	25.6	25.9	25.0	34.0	32.0	31.0	32.0	30.0	56.0
	Ja	23.2	26	27.1	23.7	23.8	22.5	30.0	18.0	17.0	29.0	30.0	40.5
	A	25.4	28	26.5	26.6	26.6	26.0	36.0	17.0	18.0	34.0	32.0	41.0
Ege U. Hosp.	Ju	25.9	26.2	26.3	26.6	26.2	25.9	49.3	53.5	49.1	47.5	47.4	52.8
	O	27.0	27.0	27.1	26.3	26.0	26.0	50.0	45.0	35.0	60.0	50.2	60.0
	Ja	22.0	29.3	30.2	29.2	28.4	28.0	50.5	57.3	60.2	55.0	48.0	47.5
	A	27.0	27.8	27.8	27.0	26.1	26.1	36.0	35.0	34.0	33.0	32.0	33.0
Celal Bayar U. Hosp.	Ju	25.7	a	a	26.2	27.0	a	54.0	a	a	a	a	a
	O	22.3	a	a	21.0	21.2	a	50.3	a	a	50.1	50.5	a
	Ja	25.2	a	a	22.3	22.0	a	48.2	a	a	46.6	46.4	a
	A	22.0	a	a	19.6	20.6	a	46.0	a	a	50.0	46.0	a

U University, Ju July 2012, O October 2012, Ja January 2013, A April 2013, 1 level 1 care unit, 2 level 2 care unit, 3 level 3 care unit, 4 inner corridor, 5 milk storage room, 6 isolation room (intensive care)

^a There is no such place in the Celal Bayar University Hospital

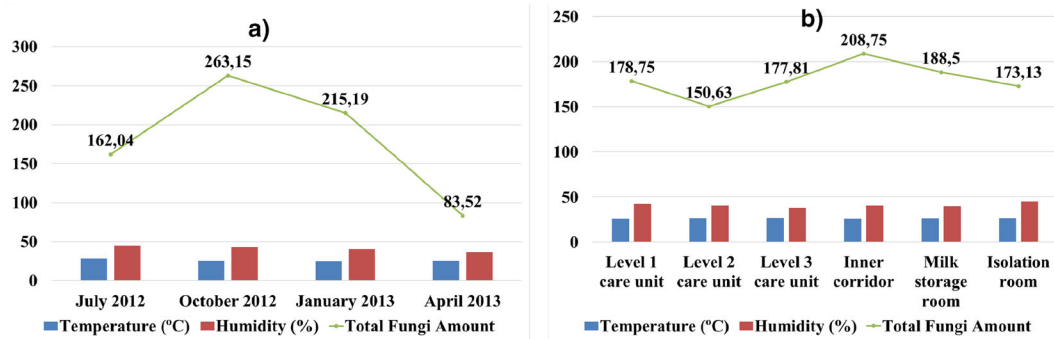


Fig. 2 Statistically seasonal differences for airborne fungi amount in sampling seasons (a) and points (b)

sampling points (Fig. 2b). A relative humidity in excess of 45–50% supports the growth of fungi in indoors (WHO 2009). This is indicating that already room conditions have need to check in respect of fungal pollution. According to the results of the relationship analysis between concentration of

airborne fungi and temperature—relative humidity, there is no statistically significant relationship between total filamentous fungi amount and temperature ($p > 0.05$). Figure 3 shows the relationship between relative humidity (%) and concentration of airborne fungi during each season. For July 2012

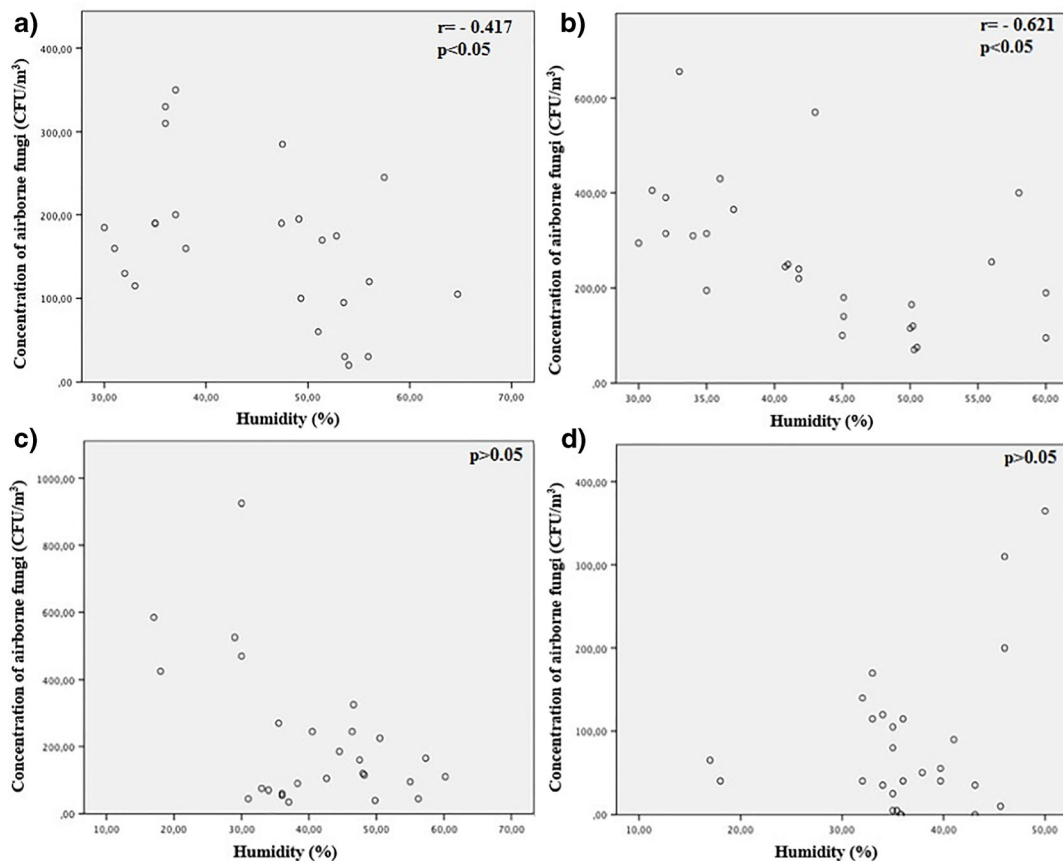


Fig. 3 Seasonal relationship between concentration of airborne fungi and relative humidity. The correlation between relative humidity and fungi concentration at **a** July 2012, **b** October 2012, **c** January 2013, and **d** April 2013

and October 2012 season, there is a negative correlation: $r = -0.417$ and $r = -0.621$, respectively. For January 2013 and April 2013 season, there are no statistically significant correlations.

According to the WHO, the fungal concentration in indoor air should not exceed 500 CFU/m³ in 1 m³ (Bogomolova and Kirtsideli 2009). However, the European Confederation Commission (CEC) declared that 2000 CFU/m³ fungi in indoor air is an acceptable value and the American Industrial Hygiene Association (AIHA) has stated that fungal levels should not be higher than 1000 CFU/m³ (Choi et al. 1999). There is no doubt that these levels should be lower in the indoor environment of a hospital. However, Kocazeybek et al. (2000) stated that the fungal concentration in sterile hospital rooms should only be 5 to 10 CFU/m³. According to the second 6-month (October 2012 and January 2013) data in the present study, the peak value of fungal counts was seen in Central Anatolia (6355 CFU/m³) and followed by Thrace (4730 CFU/m³), Aegean (3495 CFU/m³/Izmir, 2125 CFU/m³/Manisa), and Marmara (2840 CFU/m³) region as annually (Fig. 4a). These results demonstrate that the investigated newborn

units have an excessive fungal mass and it has quietly over of recommended levels (Kocazeybek et al. 2000; Ross et al. 2004).

There may be many sources of excessive fungal mass such as open windows, doors, human activities (hospital staff, visitors, faculty, students), food, and vegetation of the region. Of the investigated hospitals, only Ege University Hospital in Izmir city in the Aegean region has a ventilation system with HEPA filters. Central Anatolia, Thrace, and Marmara use open window natural ventilation systems. Although Istanbul University Istanbul Medical Faculty Hospital (41° 01' 01.62" K, 28° 56' 03.08" K) from Marmara region uses natural ventilation, there is weak vegetation in the environment of this hospital. Some studies indicate that the indoor air fungal concentration of hospitals is under the effect of ventilation conditions (Halem Khan and Mohan Karuppayil, 2012; Jung et al. 2015) and contribution of vegetation (Awad 2005; Lympelopoulou et al. 2016).

The highest annual total fungal count was observed in corridors (22%) followed by milk storage rooms (19%), level 1 care units (18%), level 3 care units (15%), isolation rooms (intensive care) (14%), and level

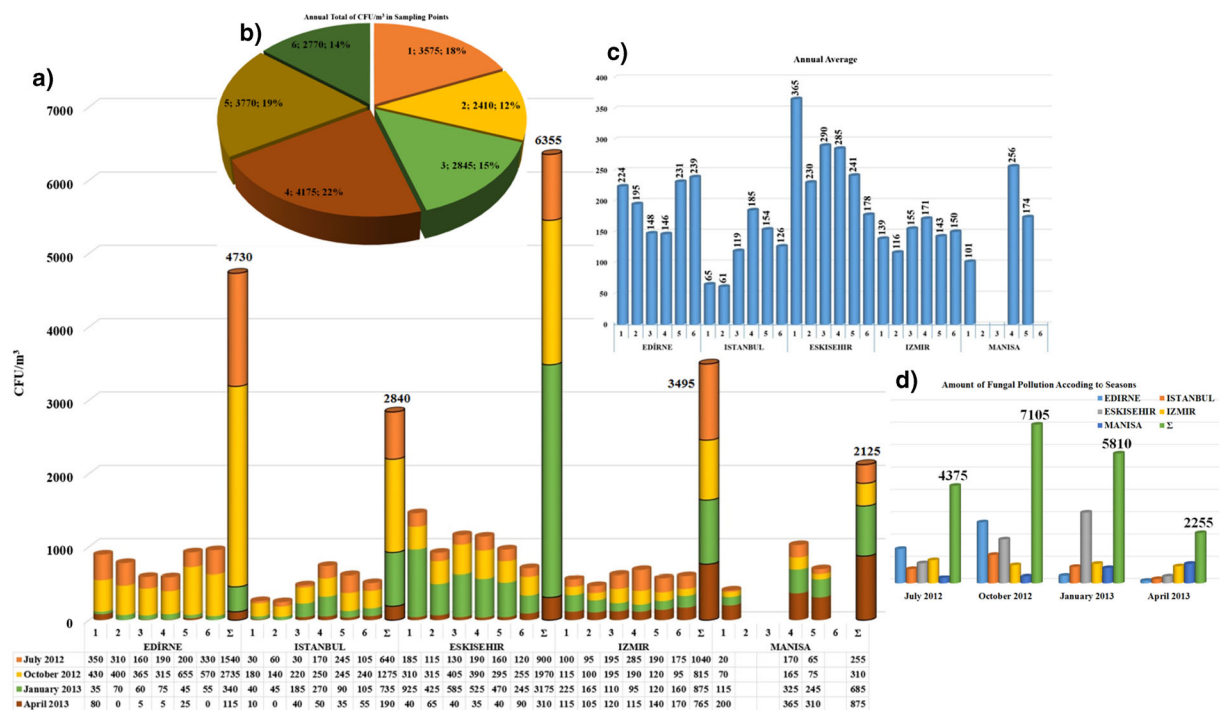


Fig. 4 Fungal colony numbers in individual working areas (a) and total sampling points (b) together with annual average (c) and seasonal CFU/m³ (d). For each panel, 1 = level 1 care unit, 2 = level 2 care unit, 3 = level 3 care unit, 4 = corridor, 5 = milk storage

room, and 6 = isolation room (intensive care). Fungal sampling was not performed on levels 2 and 3 care units or isolation rooms in Manisa City because the Celal Bayar University Hospital does not have these designated spaces

2 care units (12%) (Fig. 4b). Average annual fungal counts revealed that the level 1 care unit in Central Anatolia had the highest fungal pollution (365 CFU/m³) of all the indoor environments tested (Fig. 4c). The average annual values from other sampling points in the same region were also found to be noticeably high followed by values from the Thrace, Aegean, and Marmara regions, respectively (Fig. 4c).

When considering the seasonal levels of fungal pollution, October 2012 (7105 CFU/m³) showed the maximum fungal pollution followed by January 2013 (5810 CFU/m³), July 2012 (4375 CFU/m³), and April 2013 (2255 CFU/m³) (Fig. 4d). The relative abundance of allergenic species peaks in the fall, whereas the relative abundance of pathogens is highest in the spring (Yamamoto et al. 2012). Other studies conducted in Turkey found high counts of allergenic species during the summer (Çetinkaya et al. 2005; Topbas et al. 2006; Okten and Asan 2012; Karalti and Colakoglu 2012; Fang et al. 2015).

The mean number of fungi in the air of different areas of the five hospitals investigated varied between 0 and 925 CFU/m³ (Fig. 4a). Fungal counts were between the 0 and 655 CFU/m³ for Thrace region (Edirne), 0–270 CFU/m³ for Marmara region (Istanbul), 35–925 CFU/m³ for Central Anatolia (Eskisehir), and 95–285 CFU/m³ and 20–365 CFU/m³ for Aegean region (Izmir and Manisa) (Fig. 4a). It is understood that high air fungal pollution levels pose a health risk to newborns and precautions should be taken. Furthermore, the main health risks in investigated newborn units are not only limited with fungal pollution mass but also fungal biodiversity which is quite conspicuous.

In total, 2593 culturable airborne fungi were isolated from 108 indoor air samples collected from newborn units. After morphological examination for each species, representative isolates were selected according to species differences and 370 isolates were totally selected for molecular studies. DNA sequencing results showed that the 370 isolates represented 109 species from 28 genera. The most commonly isolated species belonged to the *Penicillium* genus [130 strains (35.14%), 31 species (28.44%)] and *Aspergillus* genus [94 strains (25.40%), 30 species (27.52%)] (Table 2). These two genera have been observed as the most prevalent in the air by many authors (Dagenais and Keller 2009; Saracli et al. 2007). In addition to these genera, species from *Cladosporium* [65 strains (17.57%), 10 species (9.17%)], *Alternaria* [23 strains (6.22%), 4 species

(3.67%)], *Talaromyces* [10 strains (2.70%), 7 species (6.42%)], *Eurotium* [3 strains (0.81%), 1 species (0.92%)], and other genera [45 strains (12.16%), 26 species (23.85%)] were identified. While many of these airborne fungi are not always pathogenic, they can cause illness in sensitive or immune suppressed individuals (Kalkanci et al. 2006). Newborns have an immature immune system: they lack mucocutaneous barriers and have relatively low B and T cell numbers and phagocytic functions and are therefore prone to infections caused by fungi (Rowen 2001; Rao and Ali 2005).

Our molecular results showed that, *Penicillium chrysogenum* (34 strains, 9.19%) and *Cladosporium cladosporioides* (31 strains, 8.38%) were the most frequently encountered species. This was followed by *Aspergillus tubingensis* (20 strains, 5.40%), *Penicillium solitum* (18 strains, 4.86%), *Cladosporium tenellum* (14 strains, 3.78%), *Alternaria alternata* (14 strains, 3.78%), *Aspergillus flavus* (10 strains, 2.70%), *Penicillium glabrum* (8 strains, 2.16%), *A. fumigatus*, *Aspergillus sydowii*, and *Penicillium griseofulvum* (7 strains, 1.89%) (Table 2). For *Aspergillus* species, the total number should not be more than 5 CFU/m³ and the recommended level is 1 CFU/10 m³ (i.e., 0.1 CFU/m³) (Barbosa et al. 2012). Among the pathogenic species identified, *A. fumigatus* is the primary causative agent of human infections. This is followed by *A. flavus*, *Aspergillus terreus*, *A. niger* and *Aspergillus nidulans* (Dagenais and Keller 2009), whereas *Aspergillus ustus* (*Aspergillus calidoustus*) causes infections less frequently (Saracli et al. 2007). Among the isolated species, the high numbers of *A. fumigatus* and *A. flavus* are remarkable and important because these species are potential infectious agents in immunocompromised patients. Even considering the species by species, recorded results with present study are quite disquieting.

Another problem found in the investigated newborn units was mixed fungal populations including many thermotolerant opportunistic pathogens (Rao et al. 1996; Zorman and Jersek 2008). The identification of thermotolerant fungi during this study is important since members such as *A. fumigatus*, *Rhizopus arrhizus*, *Rhizomucor miehei*, *Rhizomucor pusillus*, and *Absidia corymbifera* can be agents of human opportunistic fungal infections (Rippon et al. 1980). To be thermotolerant fungi within designated fungal species such as *A. flavus*, *A. fumigatus*, *A. terreus*, *A. tubingensis*, *Aspergillus uvarum*, *Hamigera ingelheimensis*, *Paecilomyces variotii*, and *Rhizopus microspores* have pointed out

Table 2 List of the identified species according to multi locus genes sequences. The number of species are given in parentheses. GenBank Accession Numbers (ITS) are from KX674594 to KX674673

<i>Aspergillus</i> (94 strains, 30 species)	<i>Eurotium</i> (3 strains, 1 species)	<i>Penicillium</i> (130 strains, 30 species)	<i>Talaromyces</i> (10 strains, 7 species)	<i>Cladosporium</i> (65 strains, 10 species)	<i>Alternaria</i> (23 strains, 4 species)	Others (45 strains, 26 species)
<i>A. amoensis</i> (1) ^b	<i>E. amstelodami</i> (3) ^c	<i>P. adametzoides</i> (1)	<i>T. aurantiacus</i> (1)	<i>C. allcinum</i> (1) ^b	<i>A. abundans</i> (1) ^b	<i>Allophoma zantedeschiae</i> (1)
<i>A. calidoustus</i> (1)		<i>P. aurantiogriseum</i> (5)	<i>T. pinophilus</i> (1)	<i>C. cladosporioides</i> (31)	<i>A. alternata</i> (14)	<i>Acremonium persicinum</i> (1)
<i>A. chevalieri</i> (1)		<i>P. bialowiezense</i> (1) ^b	<i>T. purpureogenus</i> (1)	<i>C. herbarum</i> (1)	<i>A. rosae</i> (4) ^b	<i>Chaetomium globosum</i> (5)
<i>A. clavatus</i> (3)		<i>P. bilaiae</i> (1)	<i>T. radicus</i> (1) ^b	<i>C. iridis</i> (1) ^b	<i>A. tenuissima</i> (4)	<i>Chlaetospora gossypii</i> (1)
<i>A. coreanus</i> (1)		<i>P. brevicompactum</i> (5)	<i>T. ruber</i> (1) ^b	<i>C. pseudocladosporioides</i> (3) ^b		<i>Curularia spicifera</i> (1)
<i>A. ereber</i> (1) ^b		<i>P. brocae</i> (5) ^b	<i>T. sayuliensis</i> (1) ^b	<i>C. ramotenellum</i> (8)		<i>Curularia inaequalis</i> (1)
<i>A. desertorum</i> (1) ^b		<i>P. catenatum</i> (1) ^b	<i>T. wormanii</i> (2)	<i>C. sinuosum</i> (1)		<i>Curularia tsudae</i> (2)
<i>A. dimorphicus</i> (4)		<i>P. chermesinum</i> (1)	<i>Talaromyces</i> sp. (2)	<i>C. sphaerospermum</i> (3)		<i>Fusarium oxysporum</i> (1)
<i>A. fischeri</i> (1)		<i>P. chrysogenum</i> (34)		<i>C. subinflatum</i> (2) ^b		<i>Gibberella intricans</i> (1)
<i>A. flavipes</i> (1)		<i>P. citrinum</i> (8)		<i>C. tenellum</i> (14) ^b		<i>Gibberella fujiikuroi</i> (3)
<i>A. flavus</i> (10) ^a		<i>P. commune</i> (1)				<i>Geosmithia putterlickii</i> (1)
<i>A. fumigatus</i> (7) ^a		<i>P. copticola</i> (1) ^b				<i>Hamigera ingelheimensis</i> (1) ^a
<i>A. intermedius</i> (1)		<i>P. decumbens</i> (2)				<i>Nigrospora oryzae</i> (2)
<i>A. japonicus</i> (1)		<i>P. dierckxii</i> (2)				<i>Paeclomyces variotii</i> (4) ^a
<i>A. jensenii</i> (2)		<i>P. expansum</i> (4)				<i>Phanerochaete chrysosporium</i> (1)
<i>A. niger</i> (3)		<i>P. georgiense</i> (1) ^b				<i>Rhizopus microsporus</i> (1) ^a
<i>A. ochraceus</i> (5)		<i>P. glabrum</i> (8)				<i>Rhizopus arrhizus</i> (4)
<i>A. protuberus</i> (1) ^b		<i>P. glandicola</i> (1)				<i>Quambalaria cyanescens</i> (1)
<i>A. pseudoglauca</i> (3)		<i>P. griseofulvum</i> (7)				<i>Sarocladium impletum</i> (2)
<i>A. puniceus</i> (4)		<i>P. hordei</i> (1)				<i>Sarocladium strictum</i> (2)
<i>A. quadrilineatus</i> (1)		<i>P. italicum</i> (2)				<i>Sclerotinia sclerotiorum</i> (1)
<i>A. rhizopodius</i> (1) ^b		<i>P. javanicum</i> (1)				<i>Stachybotrys chartarum</i> (4)
<i>A. sclerotiorum</i> (2)		<i>P. nalgioense</i> (1)				<i>Stemphylium vesicarium</i> (1)
<i>A. sydowii</i> (7)		<i>P. oxalicum</i> (5)				<i>Stemphylium alba</i> (1)
<i>A. terreus</i> (4) ^a		<i>P. palians</i> (5)				<i>Trichoderma lixi</i> (1)
<i>A. tritici</i> (1) ^b		<i>P. polonicum</i> (4)				<i>Trichoderma longibrachiatum</i> (1)
<i>A. tubingensis</i> (20) ^a		<i>P. raisrickii</i> (2)				
<i>A. avarum</i> (4) ^a		<i>P. simplicissimum</i> (1)				
<i>A. versicolor</i> (1)		<i>P. solium</i> (18)				
<i>A. westerdijkiae</i> (1)		<i>P. sumatraense</i> (1) ^b				

^a Includes thermotolerant strains^b New records for mycobiota of Turkey^c A member of doubtful species for one fungus=one name system

serious airborne fungal pollution level in indoor air from five different hospitals newborn units in the Thrace, Marmara, Aegean, and Central Anatolia regions of Turkey (Table 2). In addition to investigation of mycological air quality and risk factors of neonatal units at five different hospitals, we also determined some new records for microfungus biodiversity of Turkey belong to *Aspergillus*, *Penicillium*, and *Talaromyces* genera (Table 2). We believe that the discovery of these new records is important data for the analysis of unknown diseases, especially nosocomial infections.

Conclusion

The current study investigated for the first time the concentration, distribution, and identity of culturable airborne fungal pollution in the newborn units of five different hospitals in the Thrace, Marmara, Aegean, and Central Anatolia Regions of Turkey. Our results show that neonatal units have high levels of fungal contamination and consequently, low air quality. The Thrace and Central Anatolia regions were found to have the highest fungal pollution followed by the Aegean and Marmara regions, respectively. Turkey has a high fungal biodiversity and climatic properties that make it ideal for fungal growth and distribution (Demirel and Sariozlu 2014). Already, determined fungal species together with new records have remarkable with not only pathogenic properties, both also illnesses in sensitive or immune suppressed individuals with insufficient immune system. This study demonstrated that mycological air quality of investigated newborn units has an important level and distribution of fungal pollution as well as medicinally effective species on patients at neonatal units. Attention to the maintenance of air quality in hospitals will improve patient's quality of life and overall public health.

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