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ORIGINAL ARTICLE

Mycoflora of the Hospital; Water, Surface and Air

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Abstract

Background: Hospital indoor environments contain a wide variety of microorganisms. Bacteria, viruses, and fungi in these environments can cause serious healthcare-associated infections in patients. The aim of this study is to show the mycoflora of the hospital by evaluating the distribution of fungal species in hospital indoor air, water, and surfaces.

Methods: Air, water, and surface were sampled for fungi at Gazi University Hospital, using active air method, water collection, and swabbing, during a five-month period in 2022-2023. A total of 22 hospital wards were surveyed; overall, 110 air samples, 66 water samples, and 45 surface samples were collected.

Results: A total of 1331 fungal colonies were isolated from indoor air, predominantly *Penicillium* (57.7%) and *Cladosporium* (31.6%). From outdoor air (n = 471), *Cladosporium* (66.9%) and *Penicillium* (22.5%) were most common. Median colony counts per 500 L of air were 11.5 indoors and 30.0 outdoors (range: 4–21.1 and 9–55.6, respectively. Fungal diversity, as evaluated by the Shannon index, peaked in March for both environments. Fungal growth was observed in 17 of 66 water samples (26.0%), with a total of 225 colonies recovered. *Penicillium* spp. accounted for the majority (85.7%), followed by *Exophiala* (8.0%) and *Aspergillus* (5.0%).Among 45 surface samples, only 3 (6.0%) yielded fungal isolates, indicating low environmental fungal burden on high-touch surfaces.

Conclusion: The findings demonstrate that hospital environments, especially air and water sources, are significantly colonized by diverse fungal genera—predominantly *Penicillium* and *Cladosporium*—highlighting the importance of routine environmental mycological monitoring to mitigate potential nosocomial risks.

Keywords: Hospital; environmental sampling; fungi; contamination; mycoflora.

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INTRODUCTION

The hospital environment serves as a reservoir of microorganisms that may be related to healthcare-associated infections (HCAIs). Inadequate knowledge and improper implementation of cleaning and disinfection protocols can cause environmental surfaces to become reservoirs for pathogens, increasing the risk of HCAIs. Ward surfaces may become contaminated by infected patients or indirectly by vehicles such as healthcare workers' hands (1-4).

Hospital indoor environments such as air, tap water, and inanimate surfaces contain a wide variety of microorganisms. Bacteria, viruses, and fungi in these environments can cause serious HCAIs in patients. These microorganisms can be transmitted from hospital indoor or outdoor air, water, soil, or environmental surfaces. Especially infections caused by resistant microorganisms lead to significant complications and even death in Intensive Care Units (ICUs). However, the clinical outcome of fungal infections also strongly depends on the host's immune status, as the majority of isolated fungal species—such as *Cladosporium, Penicillium*, and *Exophiala*—are considered low-virulence opportunists. These organisms rarely cause disease in immunocompetent individuals but can lead to severe infections in immunocompromised patients, including those with hematologic malignancies, solid organ transplants, or prolonged ICU stays. Due to the high rates of mortality and morbidity, hospital air quality and environmental surfaces must be controlled regularly to prevent these infections (5-6). The composition and concentration of airborne microorganisms in hospital indoor air has been reported to contain airborne bacteria and fungi concentrations ranged 10^1-10^3 CFU/m^3 in inpatient facilities which mostly exceed recommendations from the World Health Organization (WHO) (7).

The role of the environment in the transmission of fungi is recently defined. In recent years, many studies have demonstrated that sink drains, shower drains, and air in hospitals can serve as a reservoir of fungal infections in patients (8-9). Fungi are an important component of the microorganisms of the air and are widely distributed in soil and water (10). *Penicillium, Aspergillus, Cladosporium,* and *Alternaria* have been reported to be the most abundant genera in hospital air (11-13). Inhalation of spores of these fungi can lead to invasive fungal infections in immunocompromised patients such as elderly patients, cancer patients, and solid organ transplantation patients (14). Additionally, some studies have reported that microorganisms isolated from hospital water increase the risk of infection in immunocompromised patients (10-11). Although there are many studies on bacterial infections caused by hospital indoor environments (15-16), studies on fungi are limited in the literature. Exposure to water in healthcare environments can facilitate the transmission of waterborne fungi such as *Aspergillus* and *Fusarium*, potentially resulting in infections and even outbreaks. (17).

The aim of this study is to show the mycoflora of the hospital by evaluating the distribution of fungal species in hospital indoor air, water, and surfaces collected from different hospital wards and ICUs.

MATERIALS AND METHODS

This study was conducted in University Hospital for five months during 2022-2023. Ethical approval was obtained from Gazi University Ethics Committee on 09.03.2023/meeting no:4 (E-77082166-604.01.02-622780).

Air

Air collections were taken from hospital interiors and outdoor areas adjacent to the hospital at approximately the same time. All air collections were examined and enumerated microscopically, and airborne fungi estimates per cubic meter of air were reported for total fungal colonies. Air samples were collected using an active air sampling method with the Air Sampler® (bioMérieux) positioned 1.5 meters above the ground. Each sample involved suction of 500 L of air over five minutes at a flow rate of 100 L/min. Sampling was carried out at five different time points over five months, in alignment with WHO guidelines and EN 171340:2020 standards for air quality validation in healthcare settings (7). The air samples were collected from pediatric wards, hematology ward, oncology ward, chest diseases ward, general surgery ward, emergency service, otolaryngology ward, ICUs, orthopedic ward, respiratory diseases ward, and outpatient hall (OH).

Water

Water samples were obtained from taps and toilet-associated fixtures in inpatient units and ICUs. Specifically, toilet water samples were collected from bidet-type hand sprayers, not from the toilet bowl. To minimize transient contamination and ensure consistency, all water samples were collected after allowing the water to run for five minutes to flush out residual stagnation from the pipes. Each sample was collected in a sterile 100 ml polypropylene container. Sampling was conducted in accordance with CDC Environmental Infection Control Guidelines (18) and the Standard Methods for the Examination of Water and Wastewater, 20th Edition by the American Public Health Association (19).

Surface

The high-touch surfaces monitored for contamination were those most frequently in contact with patients, including bed lamps, door handles, phones, assistance call remote controls, bathroom faucet knobs, and light switches. Microbiological surface sampling was conducted using sterile disposable cotton swabs, which collected residual microbial cells from a defined surface area (typically 100 cm^2). These cells were then eluted from the swab tip and cultured on a suitable medium for colony counting. The swab samples were taken once in December 2022. Surface sampling procedures were based on CDC's infection control recommendations for environmental culturing in healthcare facilities (18).

Soil

Only a single soil sample was collected during the study, obtained from the area directly in front of the emergency department entrance. This location was selected as it represents the closest accessible soil zone to the hospital building, with a potential for direct interaction between outdoor and indoor environments. Other soil areas surrounding the hospital complex were intentionally excluded due to their greater distance from clinical units and lower relevance in terms of airborne fungal transmission risk to patients and staff.

The sample was taken from the top five cm of surface soil, as this layer is most likely to harbor aerosolizable fungal spores. Sampling was performed during daylight hours in dry weather conditions, and the area was exposed to partial sunlight at the time of collection.

Approximately 10 grams of soil were aseptically collected using sterile gloves and a metal spatula and placed into a sterile sterile plastic zip-bag. In the laboratory, one gram of soil was suspended in nine ml of sterile distilled water and subjected to serial 10-fold dilutions to reduce microbial load. From the highest dilution tubes, 100 μL was plated using the spread plate method onto Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) plates. Plates were incubated at 25°C and 30°C for up to seven days, and colonies were recorded daily. Fungal identification was performed using standard macroscopic and microscopic methods as per de Hoog et al. (20).

Identification and quantification of fungal colonies

Sabouraud dextrose agar (SDA) plates were incubated at 25°C and 30°C for a period of seven days, with daily observations. The resulting fungal colonies were counted and recorded as colony-forming units per cubic meter of air (CFU/m^3). Surface and water samples were processed in the same manner by culturing on agar plates, and the number of visible fungal colonies was recorded as colony-forming units (CFUs). No further analytical testing was performed at this stage (21). Fungal identification was performed based on macroscopic and microscopic characteristics, as outlined in the Atlas of Clinical Fungi by G.S. de Hoog et al (20). Additionally, some colonies and their microscopic structures were documented and archived.

Statistical Analysis

Statistical analysis was performed using the Chi-square test to evaluate the differences in the monthly distribution of fungal genera isolated from indoor and outdoor air samples. A p-value of <0.05 was considered statistically significant. In addition, alpha diversity analysis was conducted using the Shannon diversity index (H') to assess fungal diversity within the indoor and outdoor air samples for each sampling month. The Shannon index accounts for both species richness and the evenness of species distribution, providing a quantitative measure of within-sample microbial diversity. Calculations were based on the monthly colony count data for each

fungal genus. The results are presented in a separate summary table.

RESULTS

This study presents a thorough examination of the mycoflora identified in air, water, surface and soil samples obtained from a university hospital in Türkiye. A total of 13 different fungal genera have been identified, including *Penicillium, Cladosporium, Rhizopus, Mucor, Alternaria, Aspergillus, Paecilomyces, Curvularia, Scopulariopsis, Trichoderma, Cryptococcus, Exophiala*, and *Monilia*.

Air

A total of 22 hospital wards were surveyed, and 110 air samples resulted in the growth of 1802 mold colonies. A total of 95 indoor air samples were collected, each sampling 500 liters of air. Across all samples, a total of 1331 mold colonies were isolated, corresponding to an average of approximately 14 fungal colonies per sample (i.e., per 500 L of indoor air). Similarly, 15 outdoor air samples yielded a total of 471 colonies. The fungal presence rate was 93.8% in the outdoor air samples collected, while 6.7% of them showed no mold colonies on culture. The mean colony count was 31.4 for every 500 L outdoor air sample. The fungal genera in indoor and outdoor air were more or less homogenous; *Penicillium, Cladosporium, Aspergillus, Rhizopus, Paecilomyces, Curvularia,* and *Alternaria* spp. colonies were isolated from air samples. Air samples were collected five times during the study period.

A comparative analysis of fungal genera isolated from indoor and outdoor air samples over five months revealed distinct distribution patterns. The most frequently detected genus was *Penicillium* (n = 768; 57.7%), followed by *Cladosporium* (n = 421; 31.6%) and *Aspergillus* spp. (n = 97; 7.3%). Other genera such as *Paecilomyces, Alternaria, Rhizopus, Curvularia, Trichoderma, Mucor, Scopulariopsis,* and *Monilia* each accounted for less than 2.0% of the total indoor isolates. In contrast, 471 fungal colonies were isolated from outdoor air samples. *Cladosporium* was the dominant genus (n = 315; 66.9%), followed by *Penicillium* (n = 106; 22.5%) and *Alternaria* (n = 40; 8.5%). *Aspergillus* (1.5%) was less commonly identified in outdoor air ($p<0.05$). (Figure 1)

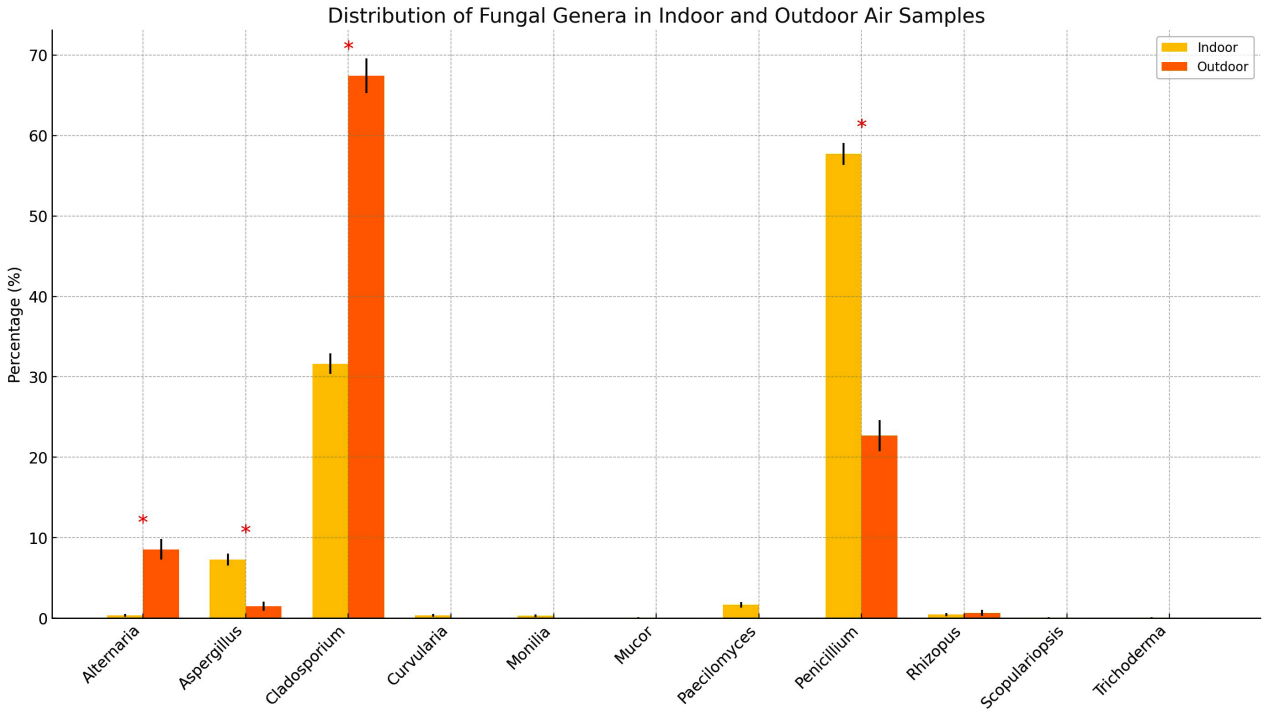

Figure 1: Comparison of Indoor and Outdoor Fungal Flora by Relative Abundance

Tables 1 and 2 provide a summary of the most frequently detected taxa in air samples collected over five months. The mycoflora was predominantly composed of *Cladosporium* and *Penicillium* species. Additionally, several other filamentous fungi, such as *Fusarium, Mucor, Rhizopus,* and *Aspergillus,* were identified. These genera are recognized as emerging fungi in medical mycology according to the World Health Organization's fungal priority list (22).

To assess the within-sample fungal diversity in indoor and outdoor air, Shannon diversity indices were calculated for each sampling month. The indoor air samples exhibited the highest diversity in March (H′ = 1.30), followed by January (H′ = 1.09), indicating greater fungal heterogeneity during these periods. In contrast, February had the lowest diversity (H′ = 0.60), reflecting a more uneven distribution dominated by a few genera. Outdoor air samples showed a similar trend, with the highest diversity observed in March (H′ = 1.07) and April (H′ = 1.03). Overall, both environments demonstrated seasonal fluctuations in fungal richness and distribution, although no statistically significant differences were found among months by Chi-square analysis ($p \geq 0.05$). (Figure 2)

Water

Hospital water samples were collected once during the study period, totally 66 samples obtained from taps and toilet outlets throughout inpatient rooms and ICUs. Fungal contamination was detected in 17 samples, corresponding to a 26.0% positivity rate. These positive samples yielded a total of 225 fungal colonies, with a mean of 13.2± 2.3 fungal colonies per 100 ml. The dominant fungal genus was *Penicillium,* accounting for 193 (%85.7) colonies, and was detected in the majority of positive samples, indicating its strong adaptation to aqueous hospital environments. *Aspergillus* spp. were present in 12 (%5.0) colonies, and both Fusarium and *Cladosporium* were each represented by a single colony. Notably, *Exophiala* spp., a genus associated with biofilm formation in plumbing systems, was also isolated with 18 (%8.0) colonies—all from tap water samples—highlighting the potential for systemic waterborne contamination.

Although the samples were not stratified by water source (e.g., tap vs. toilet), such differentiation is crucial for identifying point-source reservoirs. Additionally, no statistical comparison was made to determine whether

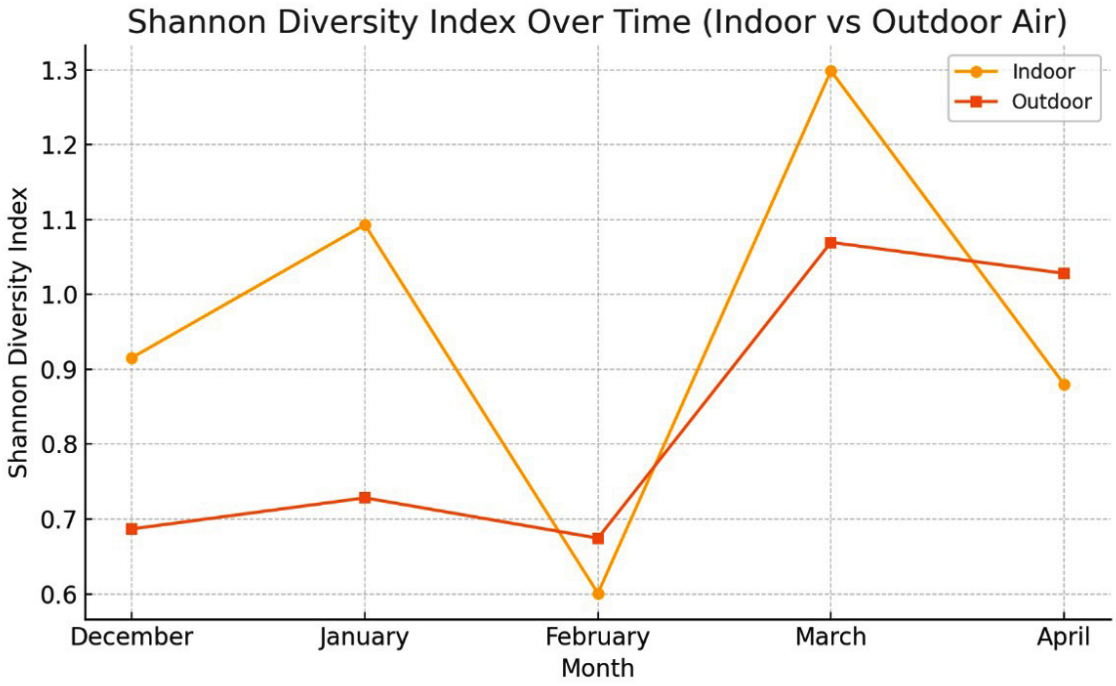

Figure 2: Shannon diversity index over time (Indoor vs Outdoor Air)

Table 1. The most recurrent strains that were detected in the indoor air over five months of the year

Fungi	December	January	February	March	April	*p* value
Alternaria	1 (0.2%)	1 (0.3%)	0 (0.0%)	2 (2.3%)	1 (0.4%)	NA
Aspergillus	10 (2.2%)	71 (23.8%)	6 (2.8%)	10 (11.4%)	0 (0.0%)	<0.05
Cladosporium	158 (34.0%)	60 (20.1%)	36 (16.7%)	26 (29.5%)	141 (53.4%)	<0.05
Curvularia	5 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Monilia	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (1.5%)	NA
Mucor	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.1%)	0 (0.0%)	NA
Paecilomyces	7 (1.5%)	1 (0.3%)	0 (0.0%)	7 (8.0%)	7 (2.7%)	>0.05
Penicillium	281 (60.4%)	161 (54.0%)	173 (80.1%)	42 (47.7%)	111 (42.0%)	<0.05
Rhizopus	2 (0.4%)	4 (1.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Scopulariopsis	0 (0.0%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	NA
Trichoderma	1 (0.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Total	465 (100.0%)	298 (100.0%)	216 (100.0%)	88 (100.0%)	264 (100.0%)	<0.05
Grand Total	1331					

*Chi-square test, NA: Not applicable

Table 2. The most recurrent strains that were detected in the outdoor air over five months of the year

Fungi	December	January	February	March	April	*p* value
Alternaria	13 (7.8%)	2 (2.2%)	2 (7.4%)	12 (10.0%)	11 (16.9%)	<0.05
Aspergillus	0 (0.0%)	1 (1.1%)	0 (0.0%)	6 (5.0%)	0 (0.0%)	NA
Cladosporium	131 (78.4%)	65 (70.7%)	22 (81.5%)	72 (60.0%)	25 (38.5%)	<0.05
Penicillium	22 (13.2%)	24 (26.1%)	2 (7.4%)	29 (24.2%)	29 (44.6%)	<0.05
Rhizopus	1 (0.6%)	0 (0.0%)	1 (3.7%)	1 (0.8%)	0 (0.0%)	NA
Total	167 (100.0%)	92 (100.0%)	27 (100.0%)	120 (100.0%)	65 (100.0%)	<0.05
Grand Total	471					

*Chi-square test, NA: Not applicable

Penicillium and *Aspergillus* co-occurred more frequently than expected by chance. Such analyses could inform whether certain fungal genera demonstrate ecological synergy or shared origin, possibly via plumbing biofilms or decaying infrastructure. Fungal diversity in water samples showed limited variability, and the dominance of certain genera suggests low evenness and potential adaptation to aquatic conditions.

Surfaces

Hospital surfaces were evaluated once. The high hand-touch surfaces were sampled by swabbing a 100 cm^2 area. A total of 45 surface samples were collected, and fungal growth was detected in 3 of them (6.0%). *Penicillium, Cladosporium* spp., and *Cryptococcus albidus* were isolated from surface cultures of our hospital. Although species-level identification (e.g., *Cryptococcus albidus*) may exceed the scope and resolution of the primary methodology employed in this study, all fungal isolates were presented at the genus level as a general rule. However, in select cases, species names were retained in the report to contribute to the extremely limited literature on fungal contamination of hospital water systems, particularly given the unexpected growth of rare genera such as *Exophiala*. The appearance of this hydrocarbon-associated yeast, in contrast to commonly encountered genera such as *Candida*, may reflect environmental or physicochemical properties specific to the sampled sites.

Soil

Soil samples in front of the emergency room were sampled. A total of 1 g soil samples were collected and cultured quantitatively. Mycoflora of the soil consisted of 600 colonies of *Cladosporium*, 55 colonies of *Penicillium,* 20 colonies of *Paecilomyces,* 10 colonies of *Cunninghamella,* and 10 colonies of *Rhizopus* species.

DISCUSSION

This cross-sectional study aimed to assess fungal contamination levels in air, water, and surfaces within a hospital environment. Ultimately, our goal was to reduce the risk of healthcare-associated infections related to environmental exposure. Hospitals should implement surveillance systems based on their size and complexity, using epidemiological data and scientific evidence. In our study, the Infection Control Committee of a 1,100-bed teaching hospital conducted environmental surveillance while the HCAI rate remained below 5.0% in 2022–2023. We monitored air, water, and surface samples to detect fungal pathogens, following current evidence-based practices. Although environmental sampling is widely accepted during outbreaks or for infection tracing, predicting HAICs solely from the presence of environmental fungi is debated. Host susceptibility remains the most important risk factor for fungal infections. Air quality varies across healthcare settings, particularly in critical care units, depending on the services provided and patient vulnerability. ICU air standards differ among countries according to ventilation and HVAC guidelines (23).

We investigated airborne fungal genera over five consecutive months. *Penicillium* was the most dominant genus throughout the study, with a peak in February (80.1%). This high prevalence may be due to its strong sporulation capacity and resistance to environmental stress, which help it persist in indoor hospital environments. *Cladosporium* was the second most frequently isolated genus, with a peak in April (53.4%). Its occurrence in both indoor and outdoor environments may account for the observed seasonal variation. *Aspergillus* showed a noticeable increase in January (23.8%), possibly influenced by higher indoor humidity or reduced ventilation during winter. The monthly distribution of *Penicillium, Cladosporium,* and *Aspergillus* showed statistically significant variation ($p < 0.05$) (Table 1), supporting a seasonal pattern. Other genera such as *Curvularia, Monilia, Mucor, Rhizopus, Scopulariopsis,* and *Trichoderma* were detected only occasionally, likely due to their lower viability in the air. *Paecilomyces* was found sporadically and showed no significant variation across months ($p > 0.05$), indicating a stable but low-level presence. Our results align with those of Demirel et al., who identified *Penicillium, Aspergillus*, and *Cladosporium* as the dominant airborne fungi in neonatal units across Turkey. Their frequent isolation of *Penicillium chrysogenum, Aspergillus fumigatus,* and *Cladosporium cladosporioides* highlights the clinical relevance of our findings, especially for indoor hospital environments. They also reported seasonal peaks in fungal counts in October and January. Furthermore, they showed that ventilation systems, humidity, and human activity significantly affect fungal concentrations—factors likely influencing our results as well (24). Sim-

ilar observations were made by Okten and Asan, who conducted year-long air surveillance in the pediatric unit of Edirne Government Hospital. They found that *Cladosporium, Alternaria*, and *Penicillium* were the most common genera, with *Cladosporium* comprising 33.6% of all isolates. Fungal concentrations were highest during summer, particularly in August, and lowest during winter. They also reported a positive correlation between temperature and *Cladosporium* levels, while *Penicillium* was more abundant during periods of high humidity and rainfall. These findings underline the impact of seasonal and environmental factors on airborne fungal loads and highlight the importance of regular monitoring, especially in units serving immunocompromised patients (25). Karaltı et al. similarly reported *Cladosporium, Penicillium, Alternaria, Aspergillus,* and *Aureobasidium* as the most common fungal genera in six Istanbul hospitals over a 12-month period. Consistent with our study, *Cladosporium* and *Penicillium* were dominant species (26). These results further confirm the strong influence of temperature and humidity on fungal growth.

Indoor and outdoor air samples showed clear differences in fungal composition. *Penicillium* was dominant indoors but less common outdoors, except in April. This pattern may reflect poor indoor ventilation or contaminated surfaces. *Cladosporium* was more prevalent outdoors, particularly in winter, reflecting its environmental origin. Its lower indoor presence may be due to filtration systems or reduced viability indoors. *Aspergillus* was frequently detected indoors but nearly absent outdoors, suggesting indoor sources such as dust, materials, or equipment. The absence of genera such as *Curvularia* and *Trichoderma* in outdoor samples might indicate their limited environmental distribution or poor aerosolization. These differences highlight how both internal and external factors shape the airborne fungal profile in hospital settings.

Fungal infections are primarily acquired through inhalation of airborne conidia. These small spores (3–5 μm) can survive harsh conditions and reach deep into the respiratory tract. *Aspergillus* species, particularly *A. fumigatus*, are commonly found in urban environments and near hospitals (27,28). Guinea et al. reported higher *Aspergillus* conidia levels in autumn and summer, increasing the risk of aspergillosis in vulnerable patients (29). Similarly, Caggiano et al. found *Aspergillus* to be the most common airborne fungus in an Italian hospital (30). Other studies also emphasize the dominance of *Cladosporium, Aspergillus,* and *Penicillium* due to their health relevance and high airborne concentrations (31-34).

Willis et al. (35) reviewed sampling in hospital environments. Water sampling commonly focuses on bacteria (19,36,37), while no fungal standards currently exist. The CDC provides general water sampling guidelines (18), which we followed. Arroyo et al. (38) documented diverse fungi in hospital water, including *Cladosporium, Penicillium, Aspergillus, Curvularia,* and *Fusarium*. In our study, we also isolated *Aspergillus, Penicillium, Cladosporium, Fusarium,* and *Exophiala* from tap water.

Environmental sampling in hospitals follows CDC recommendations (18), but due to the lack of defined fungal limits, routine culturing has declined in many U.S. hospitals. When possible, clinical and environmental isolates should be linked through molecular analysis. Periodic environmental sampling may help monitor microbial loads and evaluate air-handling systems (19). Routine surface sampling is not cost-effective for infection control, but it may serve for quality assurance. Our study aimed to document the cross-sectional fungal profile of our hospital by sampling air, water, and surfaces.

This study has several notable limitations that may have influenced the composition and quantity of the fungal isolates. First, only one soil sample was collected near the emergency department. Although it was the closest environmental source to the hospital, it may not represent the broader soil mycoflora. Future studies should include more locations. Also, *Zygomycetes,* known for rapid growth, may have overgrown other fungi, reducing overall diversity. We used only Sabouraud Dextrose Agar and Potato Dextrose Agar, without selective media like Rose-Bengal Agar, which may have limited the detection of slow-growing or pigmented fungi. Finally, we used a standard 7-day incubation at 25–30°C, which may not support thermophilic or slow-growing fungi. Therefore, our findings likely represent only part of the fungal diversity present.

In conclusion, we demonstrated the presence of various fungal genera in the hospital environment. This monitoring approach is not only essential for patient safety but also has legal importance. It supports the presence of an active environmental surveillance system, showing that potential risks are regularly assessed to protect both patients and healthcare staff.

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Abbreviations List

HCAIs: Healthcare-associated infections
ICUs: Intensive Care Units
WHO: World Health Organization
OH: Outpatient hall
CDC: Centers for Disease Control and Prevention
SDA: Sabouraud Dextrose Agar
PDA: Potato Dextrose Agar
CFUs: Colony-forming units.

Ethics Approval and Consent to Participate

Ethical approval was obtained from Gazi University Ethics Committee on 09.03.2023/meeting no:4 (E-77082166-604.01.02-622780).

Consent for Publication

It does not contain any personal data.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this paper. No financial or personal relationships have influenced the work reported in this manuscript.

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